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13. ABSTRACT *(Maximum 200 words)*

Prior research has established the ability of microwave exposure to produce a variety of biological effects including ocular changes. Investigations in our laboratory have demonstrated corneal endothelial lesions, increased vascular permeability, electroretinographic changes and structural damage. The present study was designed to verify previous results using high-peak-power pulsed microwave exposures (1.25 GHz, 1MW peak power, 1 or 16 Hz, and pulse duration of 0.5 or 10 μs) and to determine the extent of ocular damage. For this study we employed the rhesus monkey (Macaca mulatta) whose ocular anatomy and physiology is very similar to the human eye. Each subject was given a battery of clinical diagnostic tests prior to exposure and again following completion of all exposures. Each restrained subject was exposed for 4 hours, on three consecutive days over a three week period. Although some tests such as routine slit lamp examination failed to show any changes; both ERG and histopathology documented significant microwave induced retinal alteration. These results document the ability of high-peak-power pulsed microwaves to result in clinically significant ocular changes. The effects observed in this study demonstrate that under certain exposure conditions microwaves could present a human health hazard.

14. SUBJECT TERMS
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I. INTRODUCTION

The concept of using high-power microwaves to disrupt sensitive electronic systems (e.g., radar, communications, smart munitions and aircraft electronics) is not new. Recent technological and engineering advances have taken this idea from a concept to a point nearing deployment of operational weapon systems capable of generating bursts of microwave pulses, each containing hundreds of joules of energy. Projections for the year 2000 indicate a jump to 10,000 or more joules of energy/pulse and pulse lengths in the order of tens of microseconds. Also, with the advent of new microwave absorbent materials and a change in aircraft design concepts to provide a small radar cross section (stealth technology), present radar transmitters must increase power by an order of magnitude or more to maintain their present range of detection.

These changes, along with the increasing use of other microwave systems, have and will continue to increase the amount of environmental exposure to both the military and civilian population. Therefore, a research program was initiated to investigate the potential human health hazards associated with exposure to high-peak-power microwave devices that operate between 1.2 and 3.0 GHz. The most appropriate animal model for this type of study has proven to be the subhuman primate because of the overall similarity between its eye and the human eye. The primary methods of ocular evaluation were based on a combination of state-of-the-art in vivo diagnostics and conventional histological techniques, with emphasis on retinal function and ocular pathology.

II. RATIONALE

Our prior investigations have demonstrated that low-level non-ionizing microwave radiation may present a potential human health hazard under exposure conditions which may be encountered under complex battlefield situations (Kues and Monahan, 1992). Our earlier research demonstrated that exposure to low-level pulsed microwaves can cause corneal endothelial cell death and disruption (Kues et al, 1985). In the primate eye, like that of the

human, once corneal endothelial cells are lost they do not regenerate. Surrounding cells enlarge and migrate to cover the defect, and the total number of corneal cells remains depleted. In addition, iris vascular leakage (sodium fluorescein dye angiography) was found to occur in the primate model following low-level pulsed microwave exposure. This iris vascular leakage, along with choroidal leakage, has been confirmed by histologic techniques. Further histological examination also revealed possible involvement of the cerebral vasculature. Therefore, the subhuman primate model is necessary to adequately evaluate a possible human health hazard. Two species of primates were used in our previous studies -- the cynomolgus (Macaca fascicularis) and the rhesus (Macaca mulatta). Although the ocular structure is similar for both species, the observed effects seem to be somewhat dependent on the degree of ocular pigmentation with the more pigmented species being more sensitive. The present study used the rhesus monkey which has the lesser amount of ocular pigment.

The purpose of this program was to investigate the potential human health hazards associated with exposure to pulsed high-peak-power microwaves between 1.2 and 3.0 GHz using a subhuman primate animal model. The goal of this study was to establish the extent to which high-peak-power pulsed microwaves constitute a hazard to humans who may be exposed and to determine basic exposure/effect relationships.

III. OBJECTIVE

The primary objective of this research program is to evaluate the potential human health hazards associated with exposure to high-peak-power pulsed microwaves. The specific objective of this phase of the program was to verify our original findings and to determine the extent of ocular retinal damage under a variety of exposure conditions.

IV. LOGISTICS

The principal investigator and staff are well experienced in their fields. They are aware of and adhered to the guidelines established by the current "Guide for Care and Use of

Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council. We are also aware of the guidelines set forth in AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care and Public Affairs", July 1984, and DoD Directive #3216.1B, "Research and Development: The Use of Animals in DoD Programs", June 1984.

The protocol for this study required transporting animals between the Wilmer Ophthalmological Institute, in Baltimore; and the Department of Microwave Research facility in Brookville, Maryland. Transportation of the animals was conducted with the approval and supervision of the Department of Comparative Medicine, Johns Hopkins Medical Institute, and the Department of Microwave Research, Walter Reed Army Institute of Research. The animals were transported in the appropriate approved-size cages. The Brookville facility is segregated from other animal housing areas and in no way endangered the quarantine of the primates. The facility at Brookville provided the appropriate housing and care needed for animals that required overnight accommodations.

The animals procured for this program were purchased through the Department of Comparative Medicine, Johns Hopkins University School of Medicine. This institution is approved and certified by the National Institutes of Health and is certified by AALAS and AAALAC for animal care and usage.

Approval of this protocol and the use of laboratory animals was granted by the Department of Comparative Medicine, Johns Hopkins University School of Medicine and the animal use committee of the Walter Reed Army Institute of Research and the headquarters USARMDC Animal Use Review Office.

V. EXPOSURES/DOSIMETRY

Eleven subhuman primates (Macaca mulatta) were obtained for this study. Seven subjects were exposed to 1.25 GHz high-peak-power microwaves at the Walter Reed Army Institute of

Research microwave facility in Brookville. Three animals served as sham-exposed control animals, and were not irradiated with microwaves. During baseline evaluation, one of the original animals demonstrated some preexisting ocular abnormalities and was removed from the study.

The experimental subjects were exposed to 1.25 GHz pulsed radiation for 4 hours per day on three consecutive days. The exposures were repeated over a period of three weeks for a total of nine individual exposure sessions. All subjects received 1 MW peak power pulses with a repetition rate of 1 or 16 Hz and pulse durations of 0.5 or 10 μseconds (see Table 1). All exposures were performed to produce an average retinal specific absorption rate (SAR) of 4 W/kg. Since animals were restrained but could move their head slightly and because of other

TABLE I

EXPOSURE CONDITIONS

Subject Number	Exposures Weeks (days)	Exposure parameters		
		Peak Power	Repetition Frequency	Pulse Duration
21-130	3 (9)	1 MW	16 Hz	0.5 μsec
11 F[a]	3 (9)	1 MW	16 Hz	0.5 μsec
11 F[a]	3 (9)	1 MW	16 Hz	0.5 μsec
12 F[b]	3 (9)	1 MW	16 Hz	0.5 μsec
82-121	3 (9)	1 MW	1 Hz	10 μsec
82-104	3 (9)	1 MW	1 Hz	10 μsec
82-105	3 (9)	1 MW	16 Hz	0.5 μsec
82-101	3 (9)	1 MW	16 Hz	0.5 μsec
82-125	3 (9)	SHAM		
21 E	3 (9)	SHAM		
22 E	3 (9)	SHAM		

[a] Primate 11 F was exposed twice for a normal 3 week exposure protocol.

[b] Primate 12 F was not sacrificed immediately after the last exposure but was held for 1 year post exposure. Both electroretinography (ERG) and corneal endothelial changes were detected 4 months post exposure.

dosimetric limitations the actual retinal exposures probably ranged from a low of about 3.5 to high of as much as 5.0 W/kg. Exposures were conducted in the near-field in front of an open ended waveguide. This method of exposure was chosen to simulate potential personnel exposures under battlefield conditions where the near-field can extend for a considerable distance from the source of exposure. It is also in the near field situation where accidental exposures to the highest levels of microwaves exposures are likely to occur from unintended irradiation.

All exposures were conducted by personnel of Ogden BioServices Corporation (formerly ERCI Facilities Service Corporation), Gaithersburg, Maryland under contract to Walter Reed Army Institute of Research. In addition, all dosimetric measurements and parameters for a given exposure were controlled by BioServices personnel using techniques described in Attachment A and B.

VI. RESULTS

Prior to the start of microwave exposures, including sham exposure, all subjects were given a battery of clinical diagnostic tests. This procedure was followed to insure that ocular structure and function was within normal boundaries and to provide individual baseline data that could be used for comparative purposes following actual exposure. On the basis of these preexposure evaluations one animal was dropped from the study because of some abnormal findings suggestive of preexisting pathology.

At the completion of each exposure or sham exposure protocol all subjects were again given the battery of diagnostic exams. All exams performed on the sham monkeys were normal i.e., sham exposed animals showed no ocular effects from the procedures utilized in this study. The measurements performed on sham monkeys, such as electroretinography (ERG), demonstrated that ocular structure and function was within normal boundaries and remained fundamentally unchanged from their preexposure baseline values. Immediately following the final diagnostic procedure each animal was sacrificed (except subject 12 F as noted previously) and ocular tissue harvested and prepared for histopathological examination. Histologic

evaluation utilized light microscopy and in many cases, electron microscopy. Examination of ocular tissue from the sham exposed animals failed to demonstrate any cellular abnormality. It should also be noted that casual observation of both the exposed and sham-exposed monkeys throughout the experiment indicated no change in behavior that could be associated with microwave exposure.

As noted previously in Table I under this protocol not all subjects received the same exposures. Therefore, findings that are presented which are not representative of all animals will be discussed individually.

1. Specular Microscopy

Specular microscopy was performed on each animal using a Keeler-Konan SP-1 to visually examine and photograph the corneal endothelium. Both the preexposure baseline examinations and those performed following exposure demonstrated a normal corneal endothelium as seen in Fig. 1a. One exception to this finding was observed in subject 12 F when reexamined four months post-exposure. This animal demonstrated an abnormal corneal endothelium with pseudoguttata-like lesions as seen in Fig. 1b. This type of finding has been reported previously (Kues et al, 1985) from pulsed microwave exposure but was observed in only one animal in this study and showed a delayed occurrence compared to previous reports.

2. Fluorophotometry

Because of previous reports of increased iris vasculature permeability following microwave exposure some animals (11F, 12F, 21-130, 21E, and 22E) were given fluorophotometry examinations. Since this procedure can interfere with the results of some of the other diagnostic evaluations not all animals were given this test. This procedure is an automated technique for determining the amount and time course of leakage of sodium fluorescein from iris blood vessels. Detailed results of these examinations are contained in Attachment C. The three microwave exposed animals demonstrated a slight decrease in vascular permeability compared to their preexposure values while the two sham exposed subjects showed no change. A potential reason for this decrease in vascular permeability is discussed below.

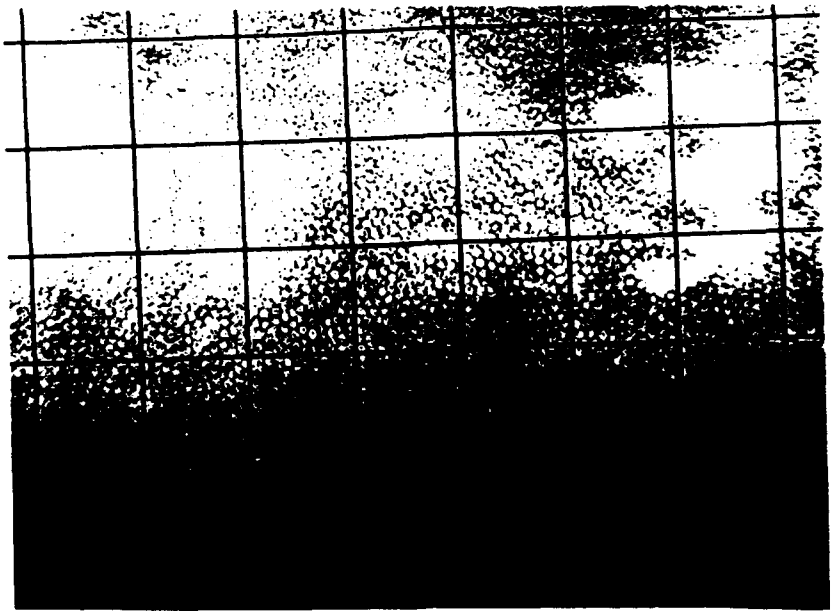


Figure 1a. Specular photomicrograph of 12 F showing normal corneal endothelium prior to exposure.

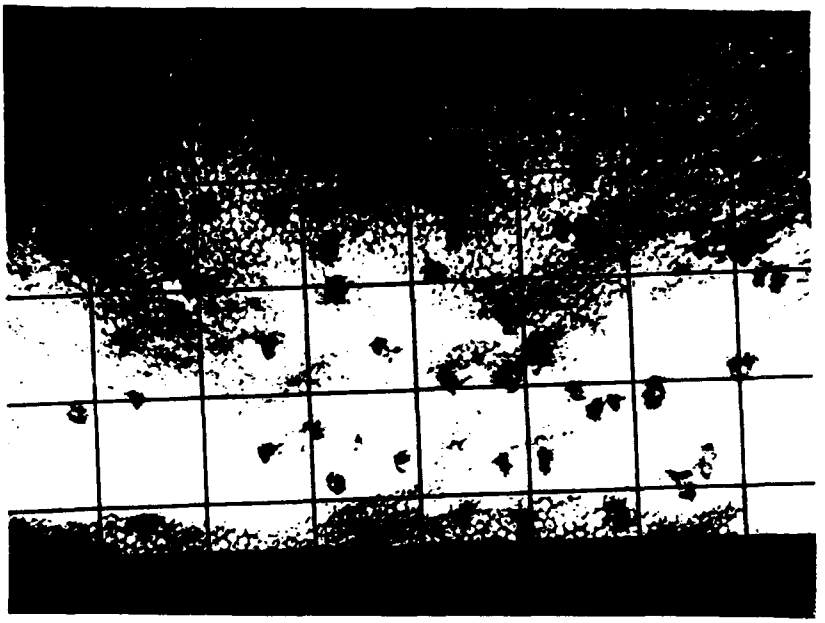


Figure 1b. Specular photomicrograph of 12 F corneal endothelium taken 4 months post-exposure showing pseudoguttata-like lesions.

3. Slit Lamp Examination

Slit lamp examination of the anterior and posterior chambers was performed on each subject to ascertain any abnormalities of the cornea, anterior segment, iris, lens, retina, etc. Although some minor irregularities were noted by the ophthalmologists performing these examinations they concluded that all eyes (both exposed and sham-exposed) were unremarkable and appeared essentially normal.

4. Fundus Examination and Photography

Fundus examination was performed and documented by color photography. Both standard color and polarized color fundus photography was performed on each subject. No differences nor abnormalities were noted in a comparison of the pre- and post-exposure photographs as seen in Figures 2a, 3a, and 4a (preexposure) and Figures 2b, 3b, and 4b (post-exposure). All sham animals also displayed normal appearance in their fundus photographs.

5. Electroretinography

All subjects were evaluated pre- and post-exposure by electroretinography (ERG) to detect functional changes (electrical) in the retinal response to light. In this study two ERG parameters were evaluated: 1. single flash response to evaluate scotopic vision (mostly rod response); and 2. flicker response to measure photopic vision (cone response) since rods are not responsive to quick flashes of light. (See Attachment D)

A summary of the post exposure ERG data is contained in Figure 5. Although the data is plotted as a percentage function of normal it should be noted that any value between 85 and 100 % is considered to be within the normal range. The summary figure contains data for five of the seven exposed monkeys. The data for 82-101 is not included because baseline values were not obtained due to technical problems. However, it is important to note that in a comparison of his raw post-exposure ERG scores to those of a normal rhesus monkey it appears that there was a substantial decrement in the cone response of this animal following microwave exposure. Subject 12 F is not included in the summary data because he had multiple ERG evaluations over a protracted period (8 months post-exposure). This subject's initial ERG

Figure 2a. Representative preexposure fundus photograph of retinal optic disc area showing normal appearance.

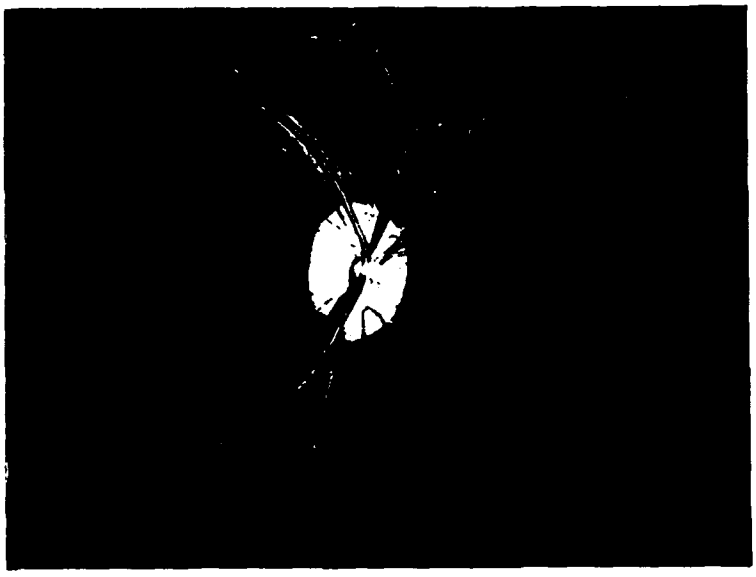


Figure 2b. Representative post-exposure fundus photograph of retinal optic disc area showing normal appearance.

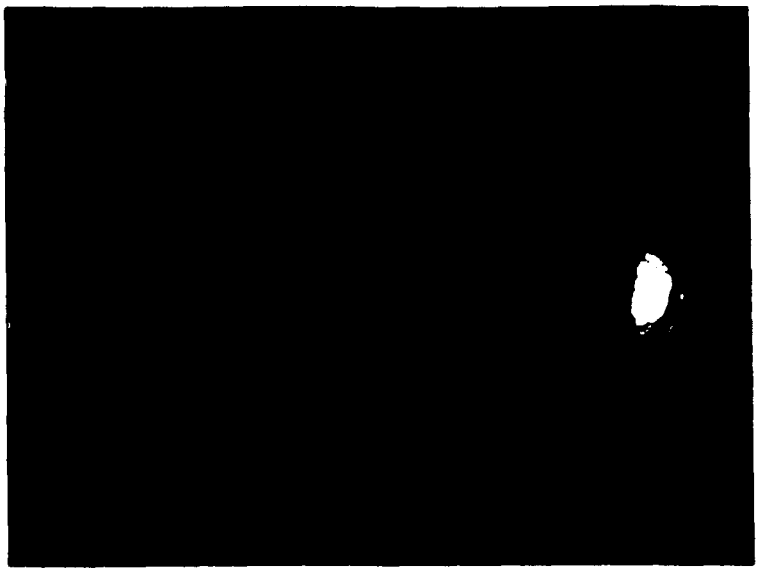


Figure 3a. Representative preexposure fundus photograph of retinal macular area showing normal appearance.

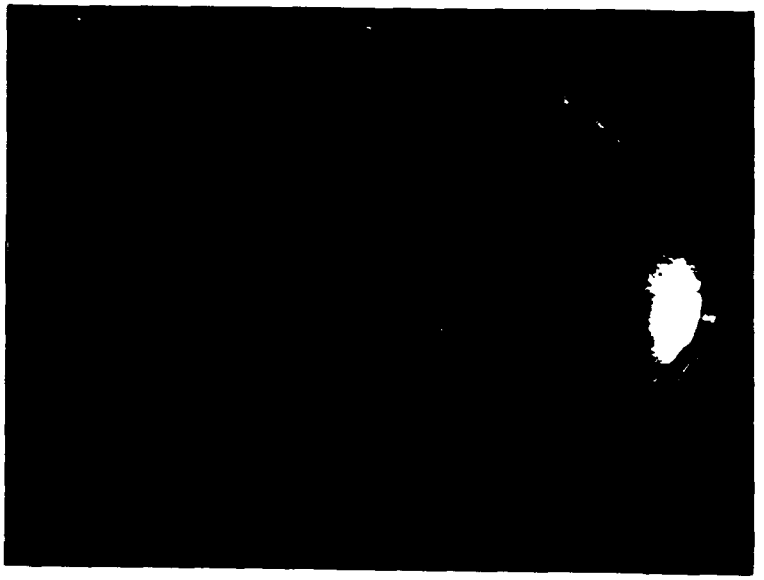


Figure 3b. Representative post-exposure fundus photograph of retinal macular area showing normal appearance.

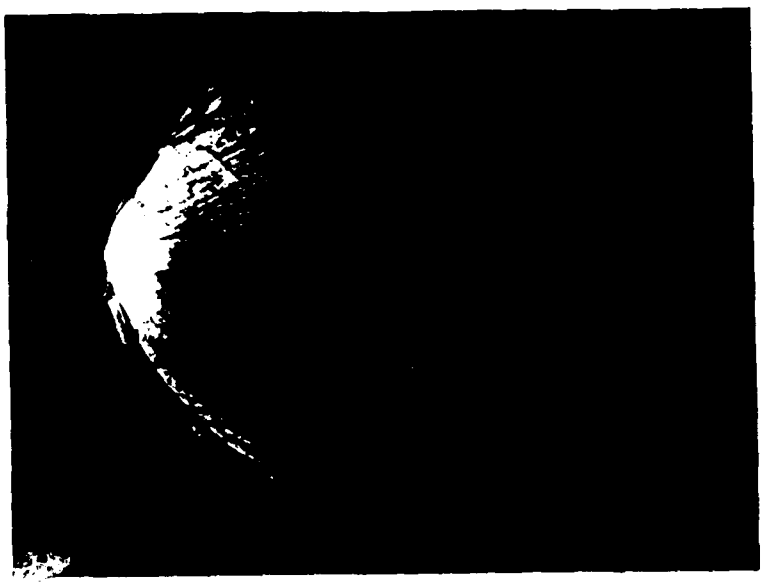


Figure 4a. Representative preexposure polarized fundus photograph of retinal macular area showing normal appearance.

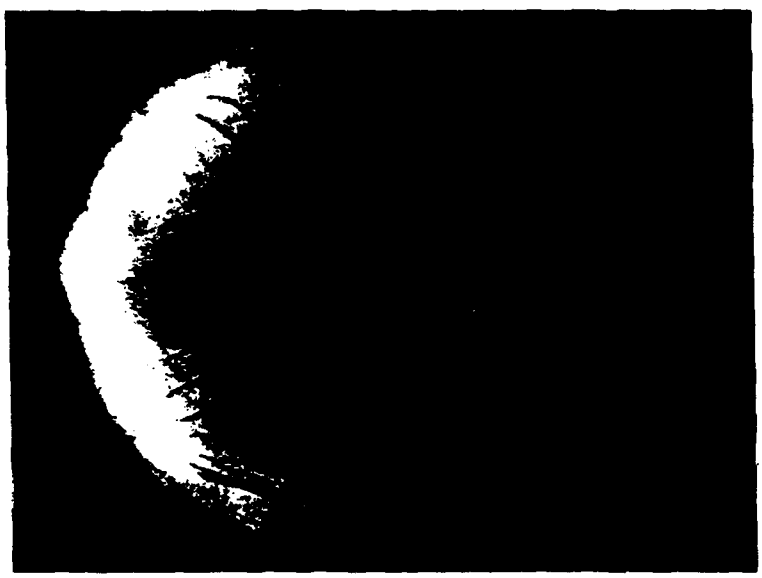
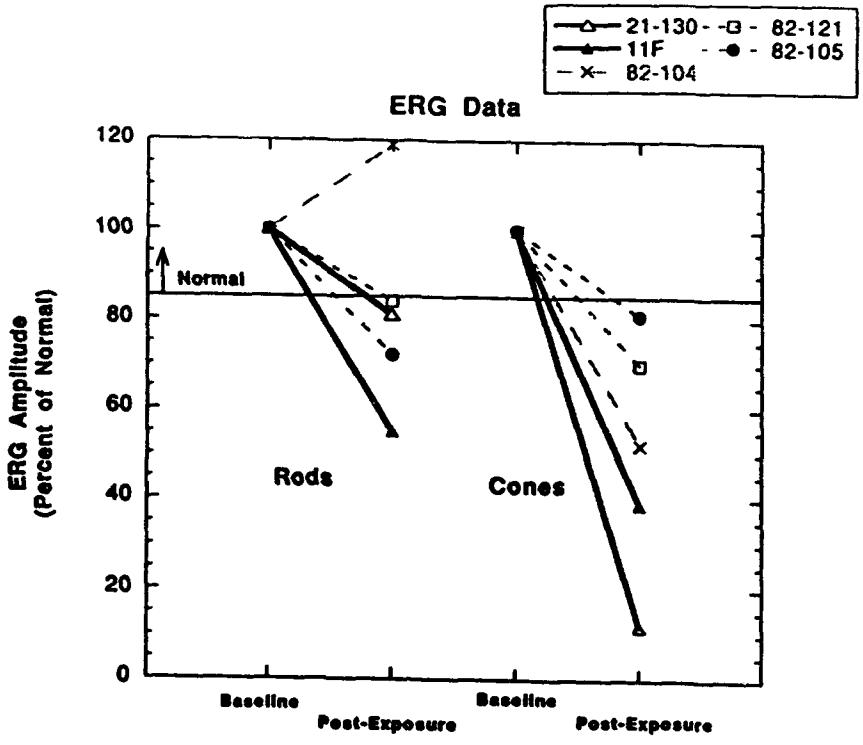


Figure 4b. Representative post-exposure polarized fundus photograph of retinal macular area showing normal appearance.

Figure 5. Summary of ERG Responses



immediately post-exposure showed very little change in amplitude of response compared to his baseline values. However, when repeat ERG evaluations were performed at 4 and 8 months post-exposure he demonstrated a 70 to 80% decrease in the cone response compared to preexposure baseline values. This decrease in cone response is very similar to the decreases noted in the other exposed animals and shown in Figure 5. Data on changes in rod function is more highly variable and as seen in Fig. 5 it appears to be decreased in only 2 subjects while showing a slight increase in one subject.

6. Histopathology

All subject eyes underwent a postmortem histologic evaluation using light or transmission electron microscopy (TEM). Detailed results of these evaluations are contained in Attachment C (subjects 21-130, 21 E, and 22 E only) and Attachment E (subjects 11 F, 82-121, 82-104, 82-105, 82-101, and 82-125). Subject 12 F's ocular tissue is currently being analyzed. A summary of histopathology impressions are listed below for each subject.

21-130 (light microscopy only) See Attachment C.

Impression:

Subject's eyes were normal.

11 F (light and TEM) See Attachment E.

Impression:

Microwave effect was observed on retinal pigment epithelium (RPE) with swelling, loss of apical villous processes, plasma membrane, and basal infoldings, cytoplasmic vacuoles, and a diminished number of mitochondria. Outer segments showed disruption of plasma membrane and discs, cytoplasmic spaces, electron dense particles, and vacuoles. Inner segments showed loss of plasma membrane. Outer nuclear layer contained cytoplasmic vacuoles. Outer plexiform layer contained swollen neurons with neuronal vacuoles. Inner nuclear layer showed extensive cytoplasmic vacuolization, loss of cytoplasmic organelles, and plasma membrane disruption. The inner plexiform layer contained swollen neurons. Ganglion cells had cytoplasmic organelle loss, vacuoles, plasma membrane disruption, and total cellular disintegration of some cells.

12 F Animal maintained for one year post exposure and histopathological evaluation currently underway.

82-121 (light and TEM) See Attachment E.

Impression:

Microwave effect showing mild disruption of the outer segments. Inner nuclear layer and ganglion cells showed mild degenerative changes.

82-104 (light and TEM) See Attachment E.

Impression:

Microwave effect with the RPE showing swelling, loss of apical villous processes and plasma membrane and ill-defined basal infoldings. Outer segments showed disruption of plasma membrane and discs, lucent cytoplasmic caps, electron dense particles, and vacuoles. Inner segments showed loss of plasma membrane. Outer nuclear layer showed nuclear swelling and chromatin dispersion. Outer plexiform layer had swollen neurons and neuronal vacuoles. Inner nuclear layer showed swollen cells with cytoplasmic vacuoles, loss of cytoplasmic organelles. Corneal endothelium had intra- and intercellular vacuoles, diminished tight junctions, and swollen mitochondria.

82-105 (light and TEM) See Attachment E.

Impression:

Microwave effects with mild disruption of the outer segments and numerous vacuoles between the photoreceptor segments and the RPE.

82-101 (light and TEM) See Attachment E.

Impression:

Microwave effect with the RPE having ill-defined plasma membrane and basal infoldings, diminished number of granules, and loss of apical processes with vesicle formation. Outer segments show disruption of the plasma membrane and discs, lucent cytoplasmic gaps, electron dense particles, and vacuoles. Outer nuclear layer showed nuclear swelling and chromatin dispersion. Outer plexiform layer contained vacuoles. Inner nuclear layer and ganglion cell layer had swollen cells with cytoplasmic vacuoles, loss of organelles, and disruption of the nuclear

and plasma membranes. Nerve fiber layer showed marked disruption of axons with vacuole formation. Corneal endothelium had intra- and intercellular vacuoles.

82-125 (light and TEM) Sham exposure. See Attachment E.

Impression:

Subject's eyes were normal.

21 E (light microscopy only) Sham exposure. See Attachment C.

Impression:

Subject's eyes were normal.

22 E (light microscopy only) Sham exposure. See Attachment C.

Impression:

Subject's eyes were normal

VII. DISCUSSION

As indicated in the introduction above the most appropriate model for assessing potential human hazards to the eye is the subhuman primate. While specific portions of the ocular anatomy and physiology can be modeled using other animal species or even in vitro systems, only the subhuman primate brings all the critical features of the human eye together in the same model. This is an especially important consideration for microwave research since even anatomical geometry and ocular thermal dynamics may play a role in any observed effects. It also appears that the unique biochemistry and physiological relationships within the primate eye may play a role in the development of microwave induced effects.

The present study was designed with the aim of demonstrating, defining, and then studying any potential ocular effect. This assessment of possible human health hazards from high-peak-power pulsed microwave exposure was designed to employ a specific average absorption rate close to that recommended as a safe exposure limit by current human exposure standards. We conducted all animal exposures in the near field at a magnitude which, given present technology, mimic those which could be encountered in a battlefield situation and thus could produce accidental human exposures. In addition, previous work on microwave induced ocular effects (Kues et al, 1992) has demonstrated that although an effect threshold does exist it can be lowered by combining microwave exposure with other factors such as drugs. Although examination of these other factors has not been included in the present study, the potential threshold lowering effect of drugs must be factored into any final conclusions which addresses a human health hazard from microwave exposure. For example, military personnel for health or protective reasons may be given a variety of drugs including such things as timolol, propranolol, and pyridostigmine. Refer to Attachments F and G.

Previous studies using specular microscopy have demonstrated corneal endothelial changes following microwave exposure. However, the present study was designed to examine primarily retinal changes with corneal endothelial alterations being a secondary consideration. Although we performed specular microscopic examinations of the corneal endothelium, lesions were noted only in 12 F and not until four months post exposure. Other subjects which were examined within 24 hours of the last exposure failed to show any gross lesions. However, when histopathology was performed endothelial effects were observed in several subjects using TEM. In the clinical setting, it is absolutely critical that the physician be cognizant of the time course of microwave induced ocular effects and they take this into consideration when planning diagnostic evaluation and when attempting an interpretation of any findings or lack there of.

A significant number of monkeys exposed and examined under this research program have revealed some retinal pathology. These pathologic changes resemble those found in other animal models (dog, rabbit) following extended exposure to hyperbaric oxygen (oxygen toxicity) It also appears to be similar to the type of retinal changes observed following ionizing radiation

or exposure to certain chemical toxins which produce oxygen free radicals. One of the possible mechanisms for explaining microwave-induced eye damage is free radical generation, so we are not surprised by the character of the observed retinal changes. Our original protocols involved the use of a general anesthetic (halothane gas and oxygen) which might produce a microwave/oxygen synergistic effect. In order to remove any ambiguity, our more recent studies, including this one have utilized restrained/ unanesthetized primate exposure protocols. The microwave effects observed in the absence of anesthetic indicate that there is no significant microwave/oxygen interaction.

A number of early very high level microwave studies have reported cataract induction in the rabbit (Carpenter and Van Umersen, 1968; Cleary, 1980). These studies demonstrated lenticular cataract formation at exposure levels of more that 100 mW/cm^2 which resulted in intraocular temperatures of 43°C or greater. An attempt to repeat these findings in monkeys was unsuccessful i.e. no cataracts were observed even at exposure levels which produced facial burns (Kramar et al, 1978). In the present study using more realistic exposure conditions with lower power levels no lens abnormalities were observed even using TEM. Thus it appears that while serious ocular effects in the subhuman primate can be induced by high-peak-power pulsed microwaves previous concerns about cataract formation and thermal damage probably do not apply to pulsed sources, such as radars, except under very unusual conditions of prolonged very high level exposures.

Previous work in our laboratory has demonstrated an alteration of iris vascular permeability following microwave exposure. In order to assess this potential effect in the present study we employed fluorophotometry to monitor fluorescein leakage from iris vessels following microwave exposure. However, because of the very real possibility that sodium fluorescein would interfere with other diagnostic techniques (such as ERG measurements) which were deemed to be of greater importance, fluorophotometry was performed in only a few animals. In the animals where fluorophotometry measurements were taken, a decrease in fluorescein leakage was observed. Other research has shown that vascular spasm and constriction can precede vasodilation and leakage. Thus the present results could represent a

timing artifact and we might actually be a measuring a phenomenon which precedes increased vascular permeability. Further study of these effects in the primate eye is warranted.

During the course of this study one subject (12 F) was not sacrificed following completion of the irradiation sessions but was held for an additional 8 months. This protocol was followed in an attempt to determine the time course of microwave induced changes in the eye. When examined immediately following the conclusion of microwave exposures this subject's ERG showed no changes compared to baseline values. However, at 4 and 8 months post-exposure this subject demonstrated a significant decrease in cone function as measured by ERG. It should be noted that this finding is reminiscent of the long-term ocular changes observed in a recent accidental human exposure case (Lim et al, 1993). It appears that ocular damage induced by microwaves can have both a delayed onset and may persist over an extended period of time. From a health perspective it appears that long-term follow up of accidental microwave exposure patients is warranted.

In the present study only one animal (11 F) was given two exposure protocols for a total of 18 exposures. It is interesting to note that this subjects had the most severe histopathological damage and also demonstrated very significant ERG changes. It can only be speculated that these effects are the result of a cumulative dose response to microwave exposure. Future research should examine the question of microwave induced cumulative effects with a much more systematic approach.

From the data obtained in this study it is apparent that microwave exposure can result in a number of ocular changes, some of which may be clinically significant. However, not all of the diagnostic test which we employed demonstrated a microwave effect. They were either not specific to the induced ocular changes or perhaps sensitive enough to detect those changes. For example, slit lamp and fundus examinations failed to detect any abnormalities following microwave exposure. On the other hand, we were able to document significant alteration of retinal function using ERG measurements and retinal structural changes were confirmed with histopathology. On the basis of the present study it appears that ERG measurements may be the

best clinical method for detection of retinal changes following microwave exposure.

VIII. CONCLUSIONS

The present study has demonstrated the ability of high-peak-power pulsed microwave exposure to cause clinically detectable and clinically significant ocular effects in subhuman primates. These findings suggest the possibility of a human health hazard under certain microwave exposure conditions including those that are within the safe exposure limits of current standards.

Under the exposure conditions employed in the present study (high-peak-power pulsed microwaves) the retina appears to be the most sensitive ocular structure followed by the corneal endothelium. From a clinical perspective the retina is clearly the most important in view of its critical role in vision. Future studies should attempt to assess the functional significance of the observed effects on the visual process.

Of necessity, the present study suffered from many limitations which should be addressed in future studies to determine the full extent of potential health hazards from accidental exposures. Clearly, such an assessment will require data from a long-term study and an examination of pulse parameters, such as the ratio of peak amplitude to pulse duration. While other studies have given us reason to believe that pulse parameters may be important variables in the production of ocular effects such parametric studies may be prohibitively expensive if performed in the monkey model and thus a more appropriate model system should be devised.

The present findings show very clearly specific diagnostic techniques must be utilized if one wishes to observe the clinical changes induced by microwave exposure. Routine examination employing standard diagnostic techniques, such as slit lamp examination and fundus photography, may not be capable of detecting ocular damage from microwave exposure.

The use of transmission electron microscopy to provide a histological evaluation of

microwave damage has revealed that retinal damage is more extensive than previously suspected. Clinical evaluation of microwave damage may need to use more specialized diagnostics techniques in order to adequately assess accidental exposures.

The results of the present experiments may help establish a better scientific basis for setting safe microwave exposure guidelines for humans.

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4. Kues HA, LW Hirst, GA Lutty, SA D'Anna, and GR Dunkelberger. Effects of 2.45 GHz microwaves on primate corneal endothelium. Bioelectromagnetics 6:177-185, 1985.

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ATTACHMENT A

Monkey and Rabbit Microwave Dosimetry for Ocular and Brain Effects Experiments

Vol. 1

Chuck Gambrill

December 5, 1988

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Suite 130
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TITLE: Monkey and Rabbit Dosimetry for Monkey and Rabbit Ocular Effects Experiments, Vol. 1

PRINCIPAL INVESTIGATOR: Henry Kues

ABSTRACT

Microwave dosimetry was performed on rabbit and monkey carcasses using the Cober transmitter at 1.25 GHz. The animals heads are placed in front of open ended W/R 650 waveguide for exposures. Measurements are taken using a Luxtron microwave transparent temperature measurement system and a computer data acquisition system.

INTRODUCTION

Monkeys and rabbits were exposed to 1.25 GHz microwave radiation using the Cober transmitter. Dosimetric measurements were done for two purposes. First, to determine the maximum transmitted power level which would not raise the animals ocular temperature above an equilibrium point. This level is the highest level that can be used without inducing microwave heating effects. The second purpose was to determine the normalized S.A.R.. The normalized S.A.R. is used to determine microwave absorption rates for animals at any power level for experiments.

EQUIPMENT

Description	Serial No.	Calibration Date
HP 435B Power Meter	2342A07284	3-30-88
HP 8481A Power Head	1550A06723	3-30-88
HP 435A Power Meter	1601A03889	3-30-88
HP 8481A Power Head	1550A06754	3-30-88
Wavetech 8502 Power Meter	1510860	-
Wavetech 16936 Power Heads	42-00613	Daily
	42-00616	Daily
Luxtron 3000	30125	-
Luxtron Probe MAM-05 and MAM-10		Daily
Bi-directional		
W/R 650 Coupler	A-97	7/7/88
HP Vectra Computer		
Data Translation DT 2801 D/A - A/D Card		
Data Translation DT 707 Screw Terminal Board		

PROCEDURE

The experimental set up is shown in figure 1. All power heads are calibrated before being used each day. The Luxtron system is also calibrated and a system check is performed. This procedure checks the Luxtron and the computer data acquisition system. The procedure is outlined in Thermometric Dosimetry Procedures as written by Howard Bassen. Temperature data is recorded using the Asystant+ data acquisition and analysis software run on the HP Vectra computer. High power exposures are

direct microwave heating can be separated from indirect heating from adjacent hot spots. Exposures last from approximately 5 to 30 seconds while the temperature rises about 2 degrees Centigrade. S.A.R.s are calculated using Asystant+ data analysis routines.

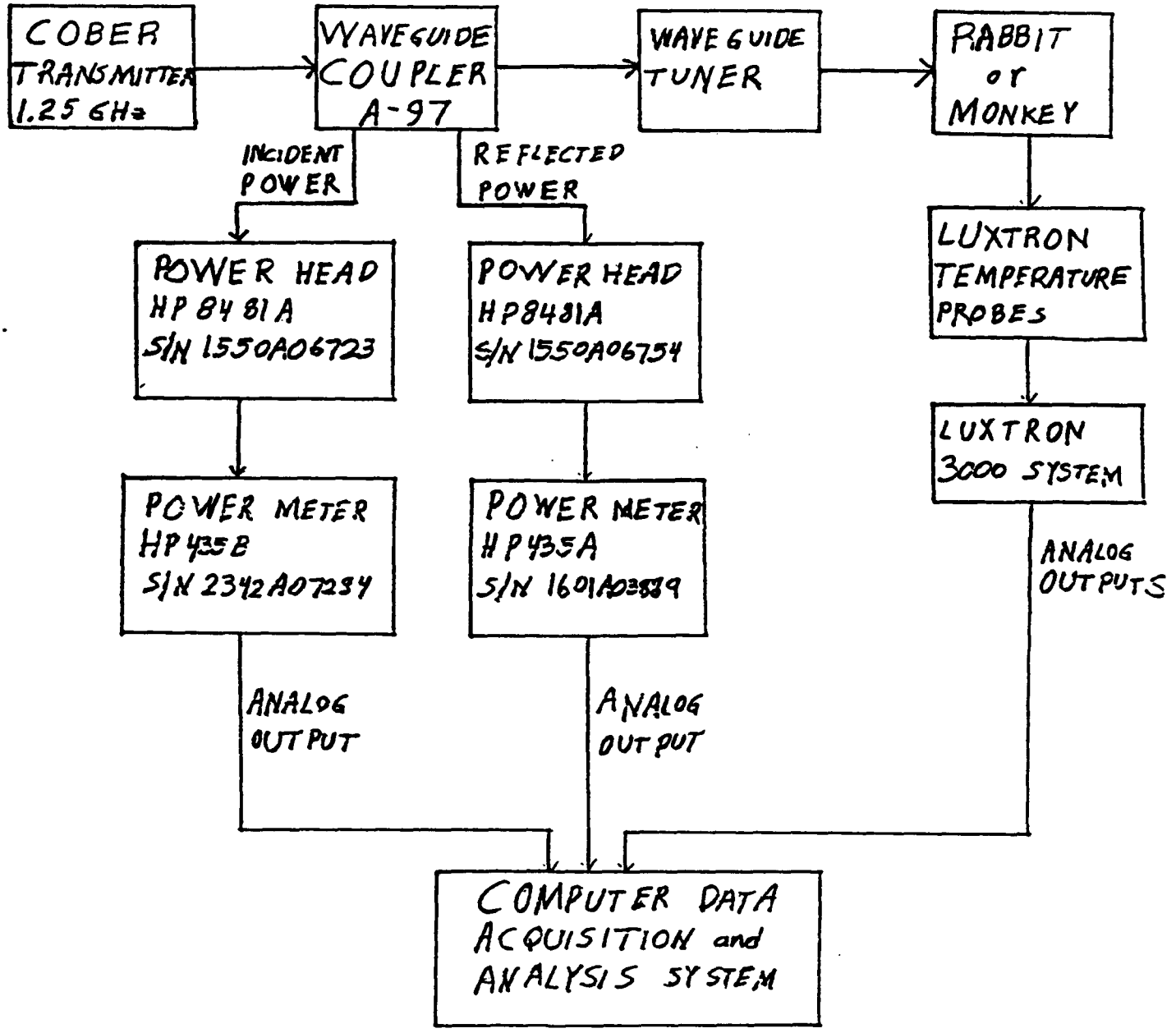

Figure 1: System Diagram

Johns Hopkins
Dosimetry
May, June and July, 1988

$$\text{Specific Absorption Rate S.A.R.} = \frac{T2 - T1}{t2 - t1} * \frac{C}{Pinc}$$

Where T1 = Centigrade Temperature 1
T2 = Centigrade Temperature 2
t1 = Time 1
t2 = Time 2
C = Specific Heat (J/Kg-Centigrade)

Specific Heats

Material	Specific Heat J/Kg-Centigrade
Eye	3510
Muscle Phantom	3640
Monkey Brain	3410

RABBIT DOSIMETRY

Figure 2 is a top view of the rabbit's position in front of the waveguide and the temperature probe positions. The waveguide narrow wall is parallel to the floor and the long axis of the rabbit's body is parallel to the waveguide narrow wall edge. E_h is the horizontal distance from the center of the incident eye to the waveguide aperture horizontal center. E_d is the horizontal distance from the incident eye surface to the waveguide aperture. The brain probe was inserted along a level tract 4mm behind the incident eye. Figure 3 is a side view. Note that E_h can not be seen here. Figure 4 is an expanded figure showing the probe sensor positions in the eye. A Luxtron MAM-05 probe was used which means all distances between probe sensors are 5mm. A is the deepest, approximately 15mm deep. B is 10mm deep and c is 5 mm deep. D is in the cornea layer. Data from D was not always

used because sometimes it was so close to the surface or just outside the surface of the corneal and did not give reliable data.

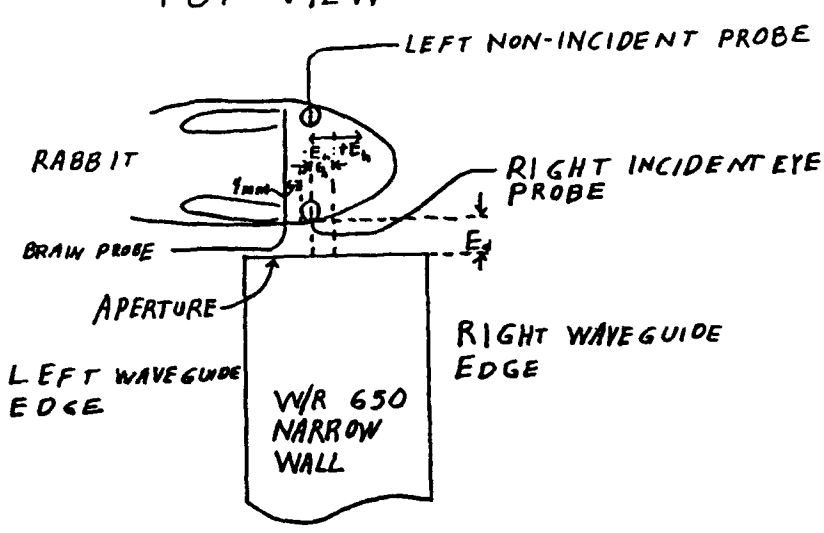

Figure 2: Top View of Rabbit and Temperature Probe Position in Front of Waveguide Aperture.

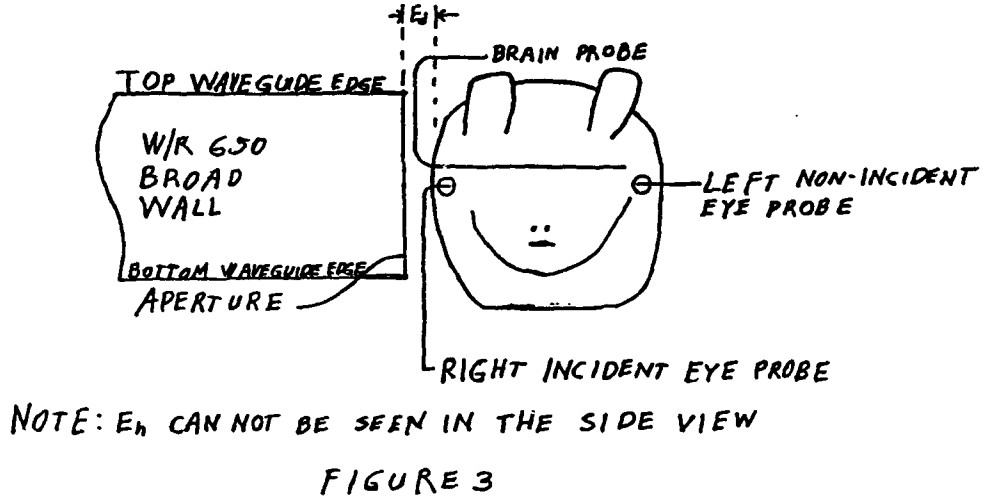

Figure 3: Side View of Rabbit and Probe Position in Front of Front of Waveguide Aperture

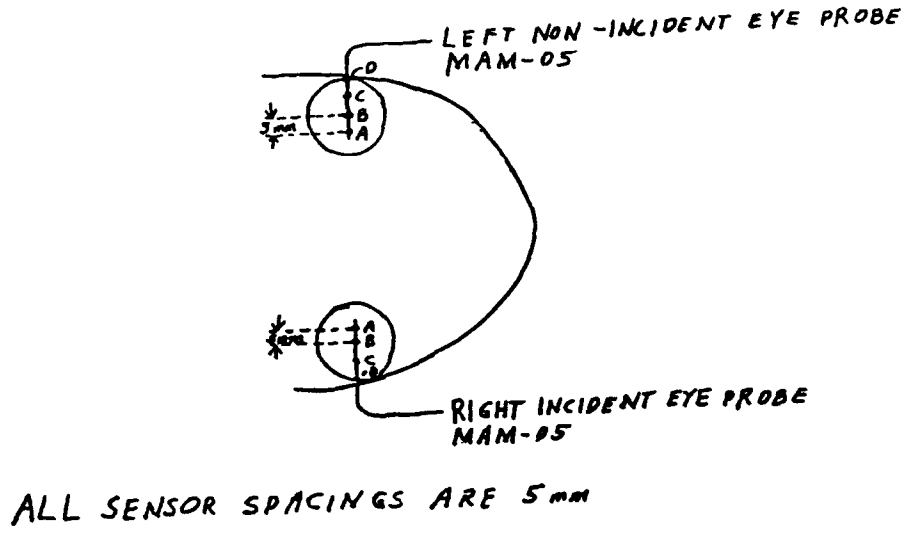

Figure 4: Expanded View of Temperature Probe Sensor Positions in Rabbit Eyes

The following is a summary of the raw data. Each exposure is given a name and date. The probe locations and SARs are given. The rabbit position is given using the dimensional variables shown in figures 2 and 3. Animal weights are given when available. Incident power is given in Watts. Reflected power was 15 dB or more lower than incident power so it was considered negligible.

RABBIT DOSIMETRY DATA

Exposure/ Date	Probe Location	S.A.R. (W/Kg/W)	Comments
HQDOS1 5/31/88	incident eye 15mm depth	3.7	Pinc = 212W Eh = 0cm Ed = 2.5cm
	10mm depth	3.0	
	5mm depth	2.9	

RABBIT DOSIMETRY DATA (cont)

HQDOS2 5/31/88	non-incident brain side	0.9	Pinc = 210W Eh = 0cm Ed = 2.5cm
	brain center	1.0	Wt:N/A
	brain center	1.0	
	incident brain side	1.1	
HQDOS3 5/31/88	brain center	1.7	Pinc = 210W Eh = 0cm
	brain center	2.8	Ed = 2.5cm Wt:N/A
	incident brain side	2.3	
HQDOS6 5/31/88	right eye 15mm depth	4.4	Pinc = 140W Eh = 0cm Ed = 2.5cm
	10mm depth	3.6	Wt:N/A
	left eye all probes	negligible	
HQDOS7 6/13/88	right eye 15mm depth	1.4	Pinc = 170W Wt = 2.4 Kg Eh = 2cm
	10mm depth	1.7	Ed = 2.5cm
	5mm depth	1.9	
	left eye all probes	negligible	
HQDOS8 6/13/88	right eye 15mm depth	2.1	Pinc = 190W Wt = 2.4 Kg Eh = 0 cm
	10mm depth	1.9	Ed = 2.5cm
	5mm depth	2.0	
	left eye all probes	negligible	

RABBIT DOSIMETRY DATA (cont.)

HQDOS9	right eye		Pinc = 229W
6/13/88	15mm depth	1.8	Wt = 2.4 Kg
			Eh = 0cm
	10mm depth	1.8	Ed = 5.5cm
	5mm depth	2.3	

The SAR of highest interest is the deepest eye SAR for Ed = 2.5cm and Eh = 0. This is shown in HKDOS1, 2 and 8. Since HKDOS8 was lower than HKDOS1 and 2 and it was done on a different day the data will be averaged as follows: 0.5 * [(HQDOS1 + HQDOS6)/2 + HQDOS8] = 0.5 * [(3.7 + 4.4)/2 + 2.1] = 3.1W/Kg/W. The brain dosimetry of highest interest is the deepest brain SARs for Ed = 2.5 cm and Eh = 0. The averaged central brain SARs from HKDOS2 and 3 are used to get 0.5 * [(1 + 1)/2 + (1.7 + 1.8)/2] = 1.6 W/Kg/W.

MONKEY DOSIMETRY

Monkey dosimetry was done in a manner similar to the rabbit dosimetry. A top view of the exposure configuration is shown in figure 5. The waveguide broad wall is parallel to the floor. This allows the monkey's eyes to be bounded by the waveguide walls if the walls were extended outward towards the monkey. Ed is the distance from the surface of the monkey's face between the monkey's eyes to the waveguide aperture. Eh is the distance from the surface of the monkey's face between the *monkey's eyes to the horizontal waveguide center. Eh was zero for this series of exposures. Other positions will be used for later exposures. The rightward direction is positive and the leftward direction is negative. The Luxtron probe sensor positions in the brain are also shown in figure 5. The Luxtron MAM-05 probe was used. They were inserted into the brain with a slight angle toward the

shoulders. Sensor D was set at the inside skull edge. approximately 20mm from the zygomatic arch. The sensors appear at 5 mm intervals. Figure 6 is a side view of the exposure system. Figure 7 is an expanded view of the eyes to show the sensor position in the eyes. Sensor D was positioned at the cornea layer. The probes were tilted upward or downward as stated in the comments section. Once again, the sensors are arranged in 5mm intervals.

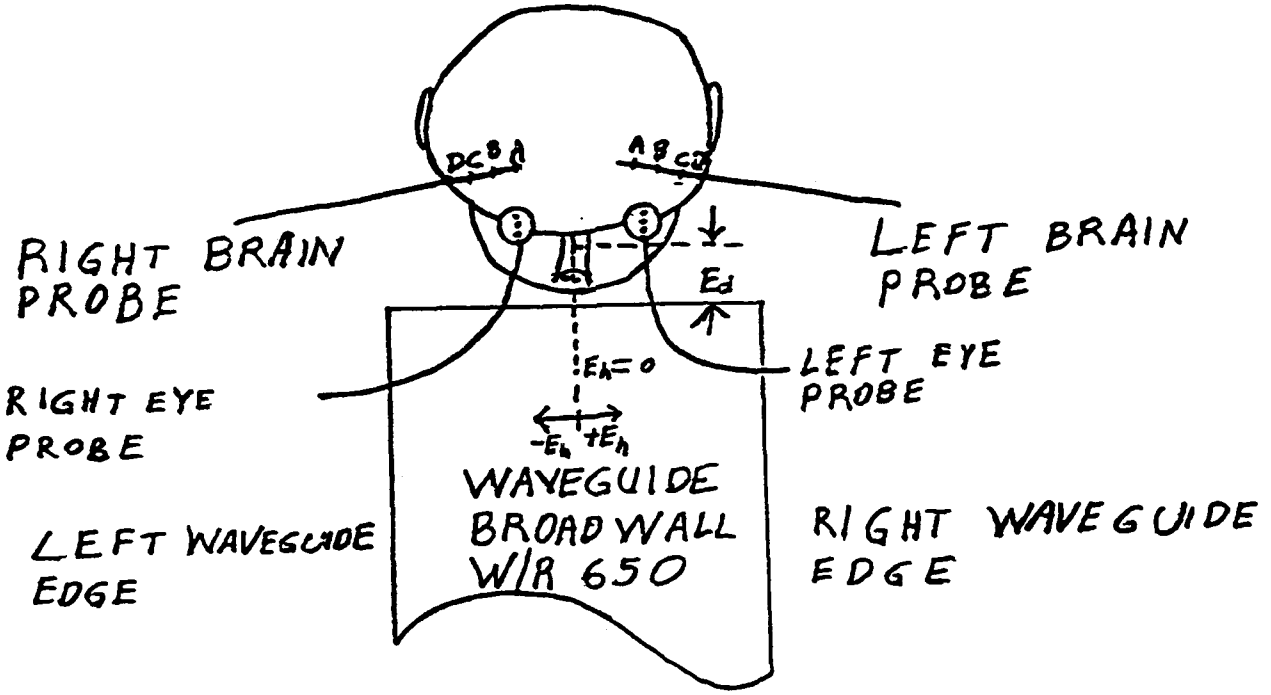

Figure 5: Top View of Monkey Exposure System

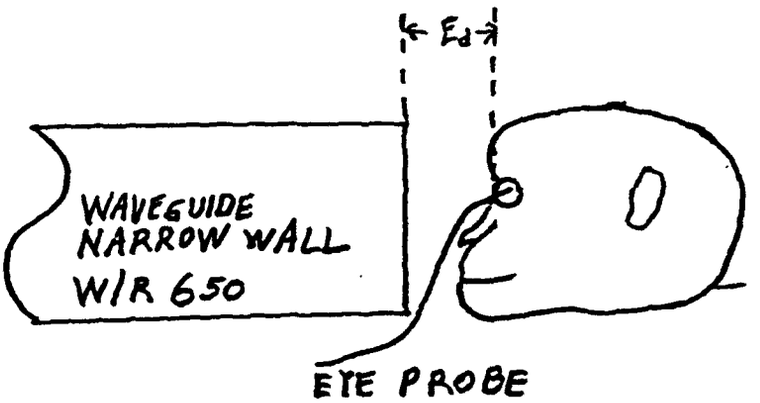

Figure 6: Side View of Monkey Exposure System

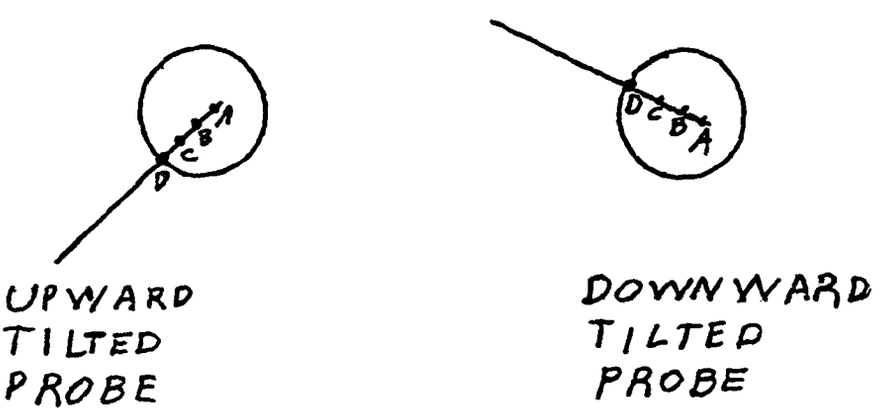

Figure 7: Expanded View of Probe Sensors in the Monkeys Eyes

ATTACHMENT B

Monkey and Rabbit Microwave Dosimetry for Ocular and Brain Effects Experiments

Vol. 2

Chuck Gambrill

December 7, 1988

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Title: Monkey and Rabbit Microwave Dosimetry for Brain and Ocular Effects, Vol. 2

Principle Investigator: Henry Kues

Abstract

Microwave dosimetry was performed on rabbit and monkey carcasses using the Cober Model 2007 transmitter at WRAIR, Department of Microwave Research. The operating frequency was 1.25 GHz. The animals heads were placed in front of open ended W/R 650 waveguide. The variance in SAR was studied as the animal position was changed. SARs were determined from temperature measurements in the animals using a Luxtron 3000 temperature measurement system and the Asystant+ Data Acquisition and Analysis System.

Equipment

Description	Serial No.	Calibration Date
HP 435B Power Meter	2342A07284	3-30-88
HP 8481A Power Head	1550A06723	3-30-88
HP 435A Power Meter	1601A03889	3-30-88
HP 8481A Power Head	1550A06754	3-30-88
Luxtron 3000	30125	-
Luxtron Probe MAM-05 and MAM-10		Daily
Bi-Directional W/R 650 Coupler	A-97	7/7/88
HP Vectra Computer		
Data Translation DT 2801 D/A - A/D Card		
Data Translation DT 707 Screw Terminal Board		

Procedure

The experimental set up is shown in Figure 1. The Cober 1.25 GHz. transmitter output power was measured using a waveguide bi-directional coupler. HP 435 power meters and HP 8481 power heads were used to measure the coupler port power. The power meter analog outputs are connected to the Asystant+ computer data acquisition and analysis system. The microwave energy travels from the coupler to the sliding short tuners arranged on a magic tee so that both electrical and magnetic field components can be tuned. The system is tuned so that the VSWR is less than 1.4. The microwave energy then travels to the open ended waveguide were the rabbit or monkey carcass is positioned for exposure. Luxtron fiber optic temperature probes are inserted into the

animals at points of interest to measure the temperature rise during an exposure. The Luxtron analog outputs are connected to the Asystant+ data acquisition system. All power heads are calibrated using the internal reference on the power meters each day before measurements are taken. The Luxtron temperature probes are also calibrated each day before measurements are taken using the Luxtron system calibration procedure with a precision platinum thermometer. A system check is performed in accordance with Thermometric Dosimetry Procedures written by Howard Bassen before and after measurements are taken. This check ensures that the Luxtron is working properly and that the Asystant+ data acquisition and analysis program is being used with the proper conversion factors.

Animals are exposed to high powers for about 15 seconds. The high power, short time exposures are used so that direct microwave heating effects are dominant as opposed to the indirect heating due to conduction from nearby hot spots. All exposures are done with the subject's initial temperature near the animals normal body temperature. SARs are calculated using Asystant+ data analysis routines.

The normalized SAR is calculated by dividing the SAR by the incident power when the exposure system is properly tuned. The SAR is calculated as follows.

$$SAR = \frac{T2 - T1}{t2 - t1} * C \ [W/KG]$$

$$\text{Normalized SAR} = \frac{SAR}{Pinc} \ [W/KG/W]$$

Where T1 = Initial Temperature in Degrees Centigrade
T2 = Final Temperature in Degrees Centigrade
t1 = initial time in seconds
t2 = final time in seconds
C = Specific Heat

The following constants were used for specific heats.

Material	Specific Heat J/Kg-Centigrade
Eye	3510
Muscle Phantom	3640
Monkey Brain	3410

In this report, whenever SAR is referred to, it is assumed to be the normalized SAR unless otherwise specified.

Monkey Eye Microwave Dosimetry
Variance with Respect to Position

This series of dosimetry was performed to investigate the variance in eye SAR as a function of monkey position in front of the open ended waveguide. The experimental set up is shown in figure 2 and 3. Figure 2 is a top view showing the probe positions and head positioning dimensions E_h and E_d. E_h is the horizontal offset of the center of the monkey nose with respect to the center of the waveguide broadwall. The positive direction is rightward and the negative direction is leftward. E_d is the distance from the center of the monkey nose between the eyes to the aperture of the open ended waveguide. Figure 3 is a side view of the same set up. Great care was taken not to move the temperature probes when the monkey was positioned between exposures. The series was done in one day to prevent day to day variance in positioning the the monkey and the temperature probes. The temperature probes were inserted only once and checked each time the monkey was moved. Muscle phantom material was used in place of the monkey eye. The actual monkey eyes had been damaged during earlier exposures. Since muscle phantom material has a specific heat comparable to eye tissue, it was considered to be a reasonable material to use for relative measurements.

Table 1 shows the right eye data with respect to monkey position in front of open ended waveguide. The position is defined by the dimensions defined in figure 2.E_d = 2" for all the exposures in table 1. The exposure is the computer data file name associated with each exposure. The depth is the temperature probe depth into the eye. The probe positions are shown in figure 2.

Table 2 shows the left eye data with respect to monkey position in front of open ended waveguide.

Right Eye SAR(W/Kg/W)

Monkey Position Ed = 2" all cases Eh Exposure	-3.25" HQDOS57	-0.5" HQDOS56	0.0" HQDOS53	+0.5" HQDOS54	+3.25" HQDOS55
Probe Eye Depth					
*Surface	-	-	-	-	-
5 mm	0.3	1.4	1.5	1.9	1.1
10 mm	0.3	1.5	1.8	2.0	1.1
15 mm	0.2	1.1	1.4	1.5	0.9

Table 1: Monkey Right Eye SAR(W/Kg/W) with Respect to Monkey Position in Front of Open Ended Waveguide

* Surface data not taken

Left Eye SAR(W/Kg/W)

Monkey Position Ed = 2" all cases Eh Exposure Probe Eye Depth	-3.25" HQDOS57	-0.5" HQDOS56	0.0" HQDOS53	+0.5" HQDOS54	+3.25" HQDOS55
Surface	0.6	2.1	2.6	2.5	0.4
5mm	1.2	3.2	2.9	2.8	0.6
10mm	1.3	3.1	2.5	2.4	0.5
15mm	1.1	2.5	1.9	1.8	0.4

Table 2: Monkey Left Eye SAR(SAR/Kg/W) with Respect to Monkey Position in Front of Open Ended Waveguide

Monkey Brain Microwave Dosimetry
Variance with Respect to Position

The series of SAR measurements shown in table 3 was taken to show the variance in the brain SAR with respect to monkey position in front of open ended waveguide. The same positioning dimensions were used as in the above eye dosimetry and are once again defined in figure 2 and 3. Data was only taken with a right brain probe.

Right Brain SAR(W/Kg/W)

Monkey Position Ed = 2" all cases					
Eh	-3.25"	-0.5"	0.0"	+0.5"	+3.25"
Exposure	HQDOS46	HQDOS43	HQDOS42	HQDOS44	HQDOS45
Probe Brain Depth					
Skull Edge	0.38	0.60	0.64	0.63	0.17
10 mm	0.25	0.46	0.52	0.51	0.19
20 mm	0.10	0.34	0.41	0.41	0.15
30 mm	0.05	0.16	0.25	0.21	0.13

Table 3: Monkey Right Brain SAR(W/Kg/W) with Respect to Monkey Position in Front of Open Ended Waveguide

Tables 1 and 2 show that the eye SAR often varies as much as 35% when moving the monkey from E_h = -0.5" to +0.5". Therefore the left and right positioning of the monkey must be carefully done in order to get repeatable SARs. Moving the monkey more right and left changes the SAR even more.

The right eye data SAR compares with the SAR determined in Vol. 1. The left eye SAR is considerably higher. This is not a major concern since phantom material was used instead of real monkey eyes. By the same token the right eye data can not be considered a conclusive confirmation of the previous data. Of particular interest is that the left eye SAR is 50% higher than the right SAR. The symmetrical configuration of the experiment suggests that the SARs would be similar. Possible sources of error are differences in the temperature probe positions between the eyes and differences between the phantom eyes. The left phantom eye weighed 11 gm. The right phantom eye weighed 10gm.

The monkey brain dosimetry data shown in table 3 indicates that the SAR varies when the monkey is moved from E_h = -0.5" to +0.5" The surface variance was about 7 % while the 30mm variance was about 55%. As expected, moving the monkey to the waveguide edge causes a larger variation in SAR. The average of all the brain SARs for this range of E_h from the surface to 20mm depth is 0.5 W/Kg/W. This is close to the 0.4 W/Kg/W determined in Vol. 1. The 0.50 W/Kg/W should be more accurate since it was determined using higher transmitted power. In general, the lower the SAR, the higher the transmitted power needed to determine it.

Conclusion

The eye dosimetry data indicates that varying the monkey position 0.5" from center causes 35% variation in SAR. The monkey must be positioned accurately to achieve repeatable eye SARs. The brain dosimetry indicates that there is not much variance on the surface of the brain, but deeper in the brain there is variance of 55%. Hence, the monkey does not need to be positioned accurately to achieve repeatable SARs near the brain surface but the positioning becomes critical deeper in the brain.

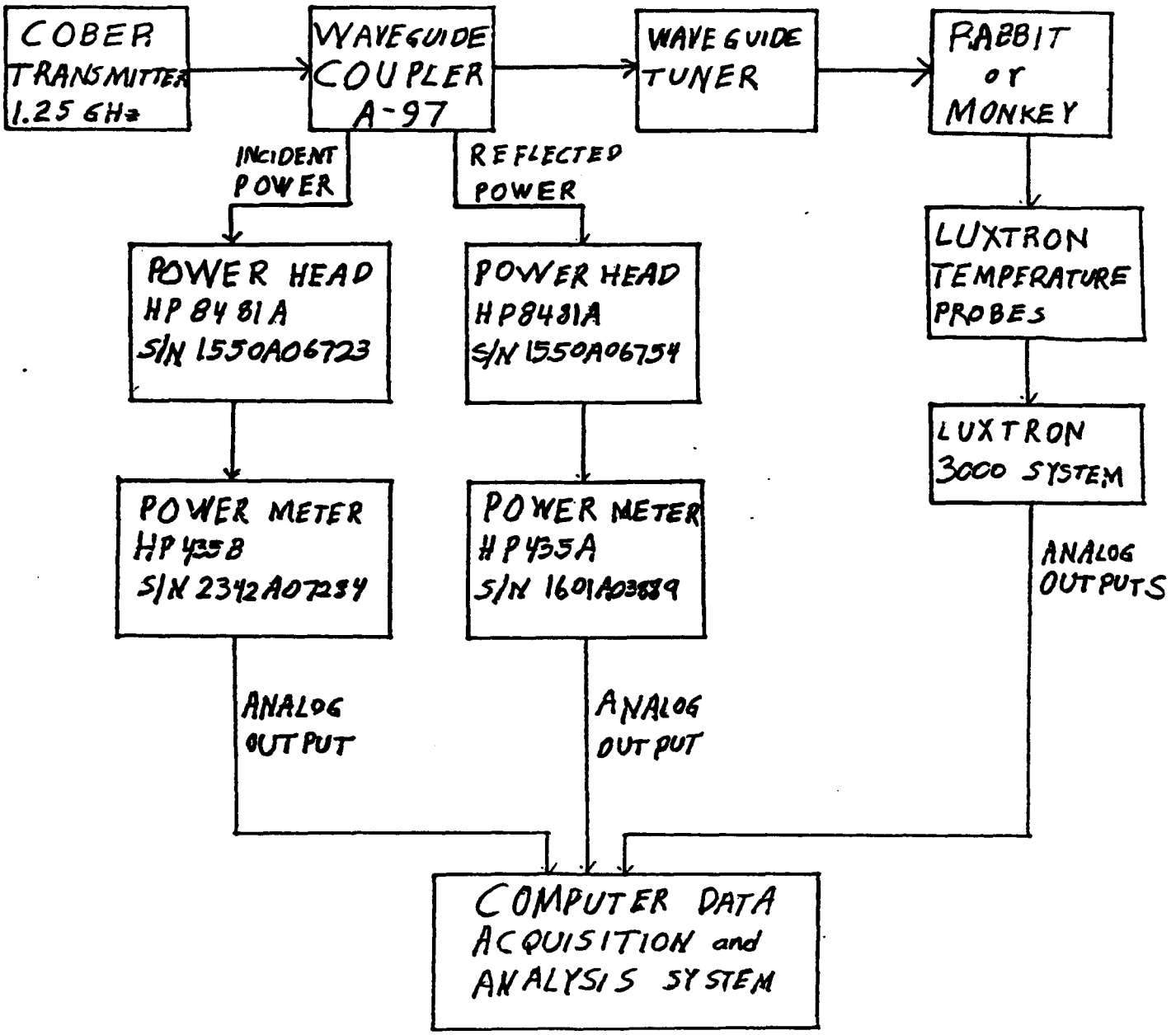


Figure 1: System Diagram

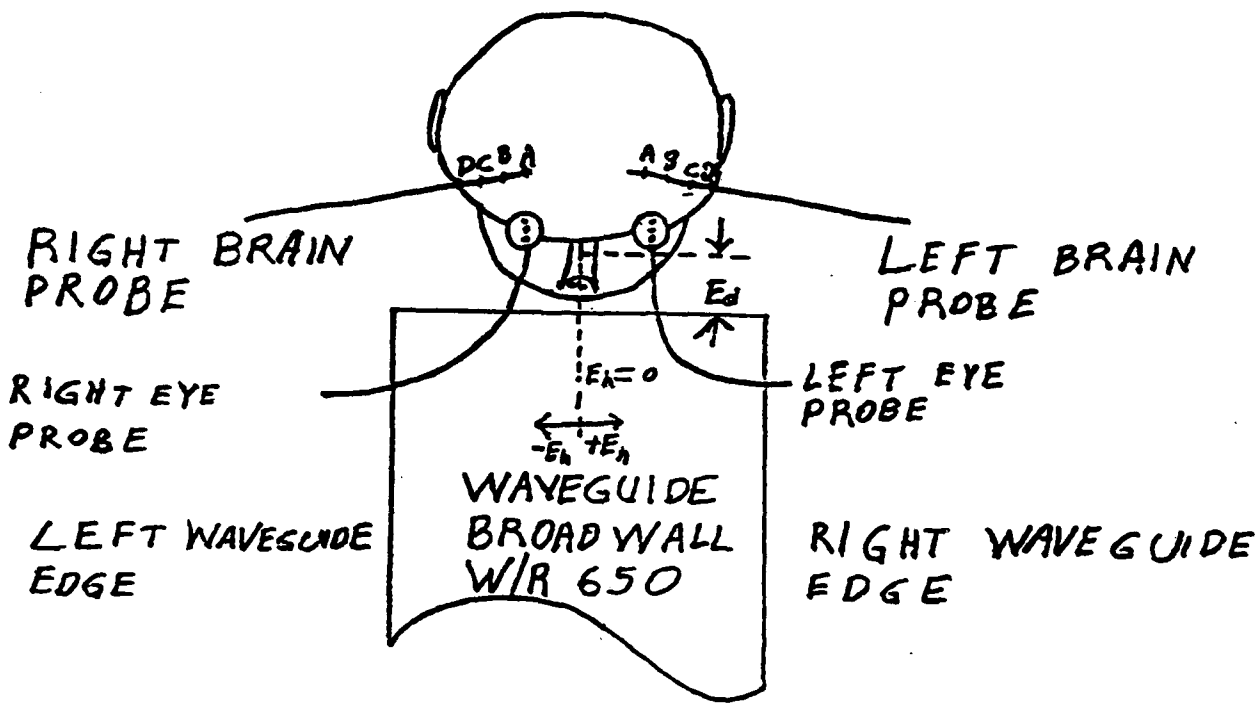


Figure 2: Monkey Exposure Set-up, Top View

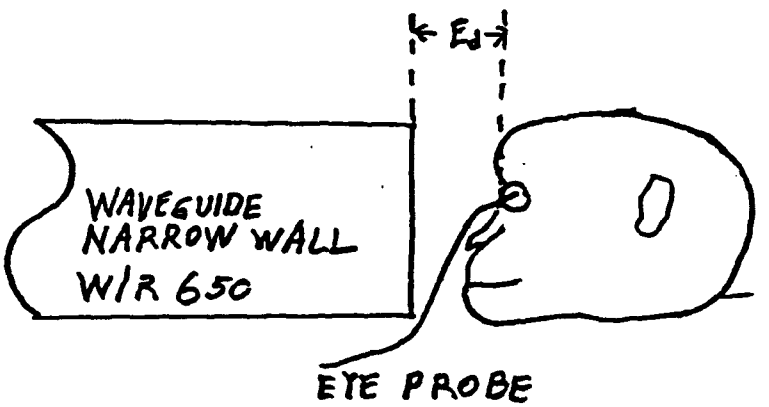


Figure 3: Monkey Exposure Set-up, Side View

ATTACHMENT C

FINAL REPORT ON MONKEYS EXPOSED TO 1.25 GHZ PULSED MICROWAVES

PERSONNEL:

Clinical Diagnostic Evaluation: Sam D'Anna
Fluorophotometric Analysis: D. Scott McLeod and Lauren Brooks
Electroretinography: Mary Johnson and Carolyn Perry
Histopathology: D. Scott McLeod and Gerard A. Lutty

1. FLUOROPHOTOMETRIC ANALYSIS OF THE BLOOD-AQUEOUS BARRIER IN EXPERIMENTAL MONKEYS

Our previous studies which examined the effects of microwave radiation on the eye have shown that experimental monkeys exposed to low level pulsed microwaves at 2.45 GHz demonstrate increased permeability of the blood-aqueous barrier to sodium fluorescein by angiography and horseradish peroxidase. In this study, we attempted to quantify blood-aqueous permeability changes with fluorophotometry. The fluorophotometer is a highly sensitive computerized system which accurately measures the fluorescein concentration in the aqueous or vitreous humour after intravenous injection. Fluorescein concentrations in the anterior chamber represent contributions of dye from the blood-aqueous barrier (ciliary body and iris vasculatures and secretion by the ciliary processes). Presented in this section of the report are fluorophotometric data from seven monkeys exposed to pulsed microwaves at 1.25 GHz and two sham exposed control monkeys. Only nine of the ten rhesus monkeys were used in this aspect of the study due to equipment failure.

Aqueous humour fluorophotometry was performed using a Coherent Radiation Fluorotron Master fluorophotometer. Aqueous humour dye concentrations were measured prior to microwave exposures (baselines), and immediately following each set of microwave exposures. For performing fluorophotometric measurements, the animal was immobilized with an intramuscular injection of 0.3 ml Ketamine, intubated and placed on Halothane/O_2 (2.5% Halothane, 97.5% O_2, 0.5 L/min). A preinjection scan was made for each eye prior to injection to determine baseline

autofluorescence. An intravenous injection of 0.3 ml of 10% sodium fluorescein was made in the saphenous vein and scans were performed at 15 min intervals for 90 min. In each case, right eyes were scanned first, followed within two minutes by the left eye. Scans of the lens and anterior chamber were analyzed using 8 point averaging . Lens fluorescence did not change significantly during the scanning period.

A. RESULTS

In all cases, a minimum of two baseline determinations per animal were obtained prior to microwave exposures. In some cases as many as three baselines were obtained. Also, equipment failures resulted in the inability to obtain several sets of data. Statistical analysis has not been performed on any of the data presented within. Data collected over the course of this study is summarized in Fig. 1. The values below and in Fig. 1 are averages of all baseline readings or averages of all values after microwave exposure.

Microwave Exposed:

19D OD. Compared with baseline data, there appeared to be a trend of decreasing blood-aqueous barrier permeability after early microwave exposure. However, the average value from all post-exposure readings was comparable to the baseline value average, as in the shams.
Baseline average @ 90 min. = 172.3 ng/ml
Microwave exposed average @ 90 min. = 177.6 ng/ml

19D OS. Data from this eye were similar to the fellow eye.
Baseline average @ 90 min. = 156.1 ng/ml
Microwave exposed average @ 90 min. = 156.4 ng/ml

13E OD. Data from this animal also suggested a slight decrease in blood-aqueous barrier permeability following microwave exposure.
Baseline average @ 60 min.* = 215.3 ng/ml
Microwave exposed average @ 60 min.* = 171.2 ng/ml

13E OS. Data from this eye was similar to OD.

Baseline average @ 60 min.* = 218.8 ng/ml.
Microwave exposed average @ 60 min.* = 153.7 ng/ml
* Note - Sixty minute scans are presented due to lack of 75 and 90 min. data following 1st set of microwave exposures.

4E OD. Following the 1st set of microwave exposures there was a significant increase in blood-aqueous barrier permeability in this animal which decreased following the next two sets to near baseline levels.
Baseline average @ 90 min. = 226.4 ng/ml
Microwave exposed average @ 90 min. = 337.4 ng/ml

4E OS. Same as OD, an increase in permeability.
Baseline average @ 90 min. = 188.8 ng/ml
Microwave exposed average @ 90 min. = 278.0 ng/ml

27E OD. Blood-aqueous barrier permeability increased significantly following the 1st and 2nd sets of exposures. Following the third set, however, values decreased but were still above baseline levels.
Baseline average @ 90 min. = 162.1 ng/ml
Microwave exposed average @ 90 min. = 243.2 ng/ml

27E OS. Same as OD.
Baseline average @ 90 min. = 151.5 ng/ml
Microwave exposed average @ 90 min. = 259.9 ng/ml

11F OD. Blood-aqueous barrier permeability decreased slightly following the 1st and 3rd set below baseline levels (2nd set readings not done).
Baseline average @ 90 min. = 183.9 ng/ml
Microwave exposed average @ 90 min. = 139.6 ng/ml

11F OS. There were no changes in blood aqueous barrier after exposure.
Baseline average @ 90 min. = 148.4 ng/ml
Microwave exposed average @ 90 min. = 153.2 ng/ml

12F OD. Blood-aqueous barrier permeability decreased below baseline levels.
Baseline average @ 90 min. = 123.6 ng/ml

Microwave exposed average @ 90 min. = 81.8 ng/ml

12F OS. Same as OD.
Baseline average @ 90 min. = 131 ng/ml
Microwave exposed average @ 90 min. = 91.2 ng/ml

21-130 OD. Blood-aqueous barrier permeability decreased below baseline levels.
Baseline average @ 90 min. = 109.6 ng/ml
Microwave exposed average @ 90 min. = 71.8 ng/ml

21-130 OS. Same as OD.
Baseline average @ 90 min. = 110.6 ng/ml
Microwave exposed average @ 90 min. = 74.1 ng/ml

Sham Exposed

21E OD. Baseline average @90 min. = 161.5 ng/ml
Sham exposed average @ 90 min. = 161.0 ng/ml

21E OS. Baseline average @ 90 min = 148.9 ng/ml
Sham exposed average @ 90 min. = 158.4 ng/ml

22E OD. Baseline average @ 90 min = 90.7 ng/ml
Sham exposed average @ 90 min. = 105.6 ng/ml

22E OS. Baseline average @ 90 min =99.9 ng/ml
Sham exposed average @ 90 min. =112.5 ng/ml

B. CONCLUSIONS

Of the seven experimental animals used in this study, only 4E and 27E demonstrated increased permeability of the blood-aqueous barrier following microwave exposure. Of the five remaining animals, 19D and 11F showed no change (11F OD had a slight decrease) while 13E, 12F, and 21-130 demonstrated

decreased permeability. The sham controls showed little or no changes following sham exposures.

Based on these data, the order of most affected blood-aqueous barrier to least affected was 4E, followed by 27E, 19D, 11F, 13E, 12F, and 21-130.

2. HISTOLOGICAL EVALUATION OF RETINAL TISSUES

All tissue was processed as described previously and embedded in glycol methacrylate. Sections were taken through the macular and foveal regions of each eye. Evaluation of retinal sections was performed by two independent observers (one in a masked fashion). Final analysis was made collectively by both observers in a masked fashion.

A. RESULTS

The most striking and substantive morphological change observed in retinas of microwave-exposed animals was the degenerative appearance of cone nuclei in the macula. The degree of degeneration was comparable in both eyes of a given monkey. The number of affected cones varied between animals. The cytological characteristics of this degenerative change were loss of basophilia of nuclear material and clumping of chromatin. In most cases the nuclear envelope appeared intact, however, the inner and outer segments of these cones were often retracted and vacuolated. In some cones, karyorrhexis (fragmentation of nucleus) had occurred. These degenerative changes in photoreceptor cell nuclei will be called collectively karyolysis in this report.

MICROWAVE-EXPOSED ANIMALS

6D, OD. Various degrees of cone nuclei karyolysis were evident within the foveal region and scattered throughout the macula. Occasional cones in nasal retina were also affected. No morphological changes were observed in rods or retinal pigment epithelium (RPE).

6D, OS. This retina had the same degree of damage as 6D, OD.

19D, OD. Karyolysis of cone nulcei was observed in central fovea and in nasal macula. No morphological changes were seen in rods or RPE.

19D, OS. Changes were comparable to 19D, OD.

13E, OD. Extensive karyolysis of cone nuclei was observed throughout the retina. The greatest number of karyolytic cone nuclei was in fovea. Karyolytic rod nuclei were also observed throughout retina but the percentage of the population affected was less than in the cone population. RPE cells had pyknotic nuclei and scant cytoplasm. Some vessels in both retina and choroid appeared partially occluded. Yellow vesicular material was observed in some vascular lumens.

13E, OS. Changes to rods, cones, and RPE cells was as extensive as in the fellow eye, 13E, OD.

4E, OD. No apparent changes were observed.

4E, OS. There were no apparent changes in retinal tissue.

27E, OD. Occasional cone nuclei karyolysis was observed in nasal retina and in fovea and macula. There were no apparent changes in rods.

27E, OS. There were more affected cones in this eye than in 27E, OD, but the overall change was quite moderate.

11F. Sacrificed later after re-exposure and tissue handled by Dr. Green.

12F. Not sacrificed.

21-130. No changes in either eye.

SHAM EXPOSED:

22E, OD. No apparent changes were observed.

22E, OS. No apparent changes were observed.

21E, OD. Occasional scattered karyolytic cone nuclei in macula.

21E, OS. There were no apparent changes in retinal tissue.

B. CONCLUSIONS

The experimental monkeys used in this study demonstrated varying degrees of photoreceptor changes which were primarily confined to cones in the macula. They were ranked in this order from most severe to least based on relative number of karyolytic cone nuclei, pyknotic rod nuclei, and change in appearance of RPE: 13E, 6D, 19D, 27E, 4E, and 21-130 (11F and 12F not analysed by these methods). In cases with extensive cone cell degeneration, the RPE cells were also affected. Of particular interest was the fact that, in general, eyes which showed an effect had very few degenerative cones outside the macular region. The exception to this was 13E which demonstrated photoreceptor cell degeneration throughout the retina. Moreover, within the macula of the moderately affected eyes, the distributional arrangement of karyolitic cone nuclei appeared in a pattern such that every fourth to fifth cone nucleus in the outermost portion of the outer nuclear layer appeared degenerative. This was best illustrated in moderately affected animals such as 27E. Detailed analysis of sections taken from different areas of retina revealed degenerative cones only within the parafoveal region, an area which has been shown to contain the highest number of blue-sensitive cones. Blue cone function unfortunately can not be measured with our ERG equipment to date. It is tempting to speculate that a specific population or subpopulation of cones were more susceptible to microwaves.

FIGURE 1:

SUMMARY OF 1.25 GHz MICROWAVE ARMY STUDY

ANIMAL	FLUOROPHOTOMETRY	HISTOLOGY (affected cells)	ERG (% of NORMAL) ROD	CONE
13E	Slight decrease permeability	Cones and rods	48%	0%
6D	N/D	Cones	44%	11%
19D	No change	Cones	58%	27%
4E	Increase permeability	No change	72% (NS)	59%
27E	Increase permeability	Few cones	119% (NS)	106% (NS)
11F	No change OS Decrease OD	Not done in JB-4	?	?
12F	Decrease permeability	Not sacrificed	?	?
21-130	Decrease permeability	No change	?	?

(NS= not significant)

ATTACHMENT D

Electroretinographic Analysis

Performed by

Mary A. Johnson, Ph.D.
Wilmer Ophthalmologic Institute
Johns Hopkins University

ELECTRORETINOGRAPHIC ANALYSIS

Electroretinography is used to detect functional changes (electrical) in retinal response to light. Two ERG parameters were evaluated in the monkeys used in this study: 1. single flash (scotopic) response which is a response of mostly rods; and 2. flicker response which measures cones because rods are not sensitive enough to respond to these quick flashes of light. Each animal was evaluated by both parameters before exposure (baseline) and then after the last exposure.

RESULTS

12F- Microwave-exposed animal with normal baseline responses and who showed very little change in amplitude of response from baseline directly after the first set of exposures. This animal was not sacrificed, but rather was re-baselined on 3/16/93. At this time, his cone ERGs measured between 20 and 30% of their previous values. ERGs were again recorded on 6/4/93. His cone responses were still reduced at this time, and were equivalent to the responses measured on 3/16/93.

11F- Amplitudes of rod responses were 55% OD and 49% OS of baseline responses. This is a significant reduction.
Amplitudes of cone responses were 39% OD and 26% OS of baseline responses. This is a significant reduction.

21-130- Amplitudes of rod responses were 81% OD and 78% OS of baseline responses.
Amplitudes of cone responses were 12% OD and 15% OS of baseline responses.

This animal had the most affected cone ERG response in this segment of the study. The reductions in the scotopic response are just significant and probably reflects the large loss in cone function. (While the scotopic response is mostly comprised of responses of the rods, it also contains some output from the cones).

82-121- Amplitudes of rod responses were 84% OD and 97% OS of baseline responses. ERGs were borderline normal OD and normal OS. Amplitudes of cone responses were 70% OD and 87% OS of baseline responses. ERGs were reduced OD and borderline normal OS.

Of the animals that showed an effect of microwave exposure, this animal was the least affected.

82-101- This animal was not baselined. However, his final cone ERGs were low, measuring 22 μV OD and 19 μV OS. These values are similar to the final cone ERGs of 82-104. Final rod ERGs of this animal were unremarkable.

82-104- Amplitudes of rod responses were 1 19% OD and 89% OS of baseline responses. These responses are normal.
Amplitudes of cone responses were 52% OD and 29% OS of baseline responses. These responses are reduced.

82-105- Amplitudes of rod responses were 72% OD and 68% OS of baseline responses. These responses are reduced.
Amplitudes of cone responses were 81% OD and 85% OS of baseline responses. ERGs are borderline normal/slightly reduced for both eyes.

In terms of cone damage, the most affected animal was 21-130, followed by 11F, 82-104, possibly 82-101, 82-121, and 82-105. 82-121 and 82-105 had marginal amounts of damage.

The results are summarized in Figure 1. The actual scans are shown in Figures 2-8.

FIGURE 1: SUMMARY OF ERG RESPONSES.

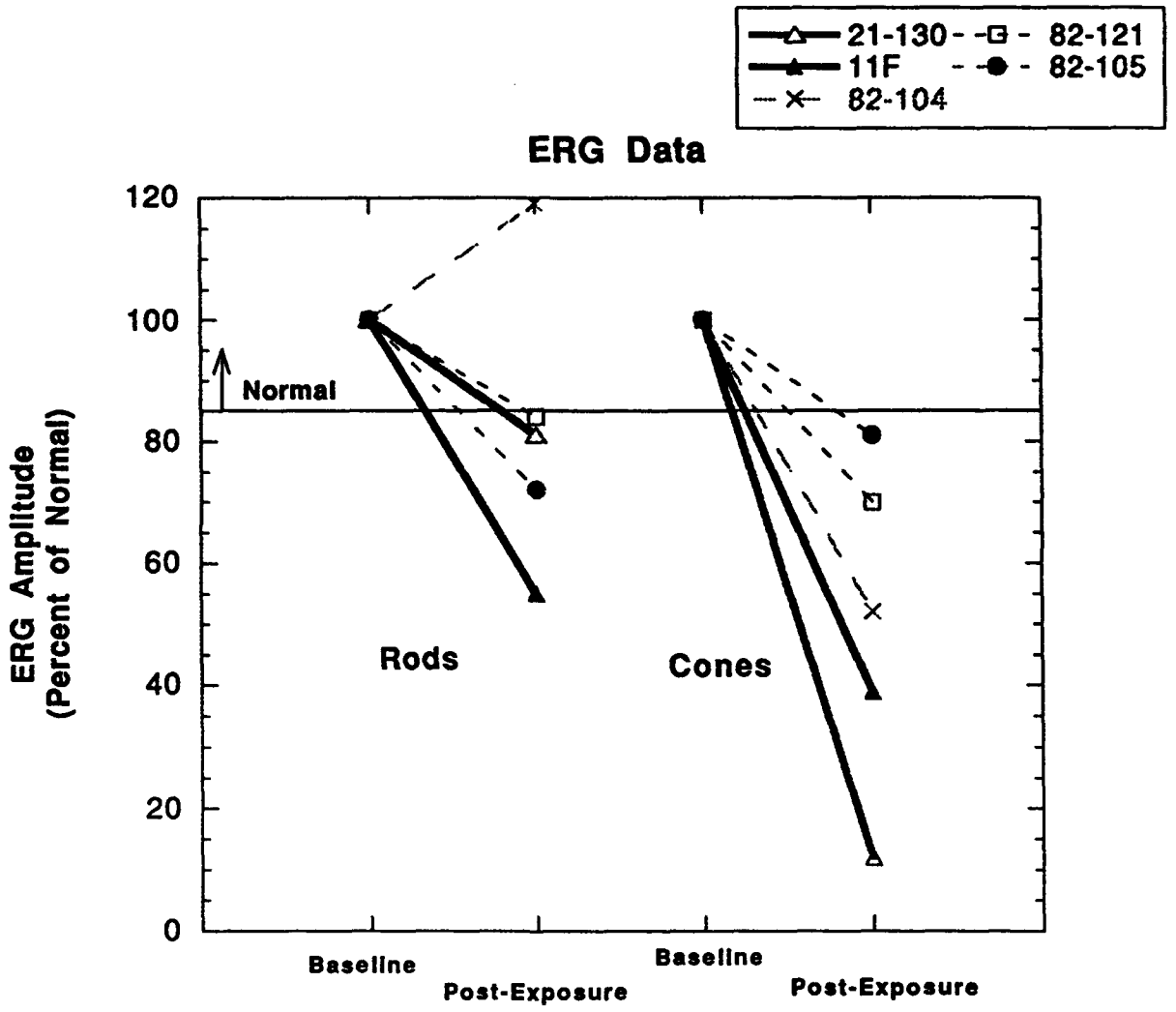
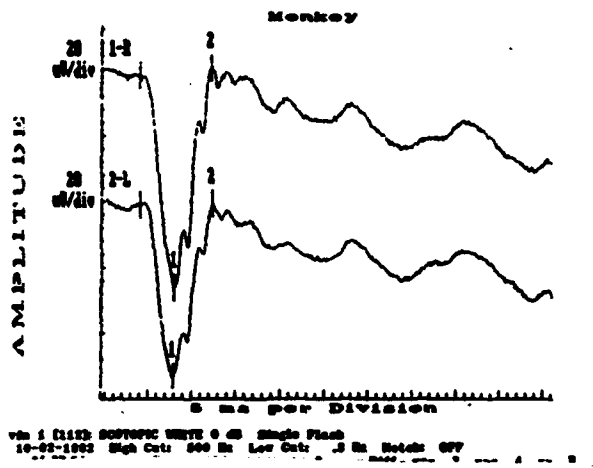
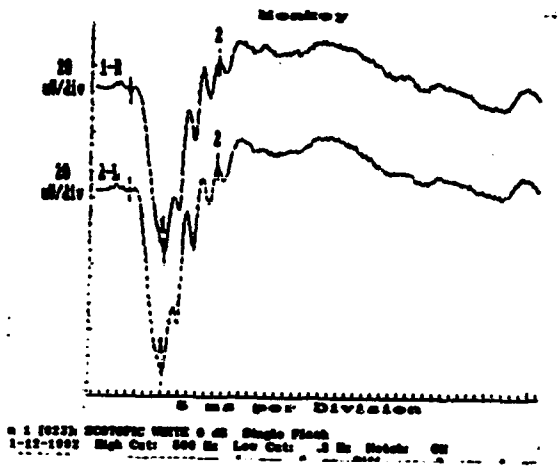


FIGURE 2: ERG RESPONSES FROM MONKEY 12F.

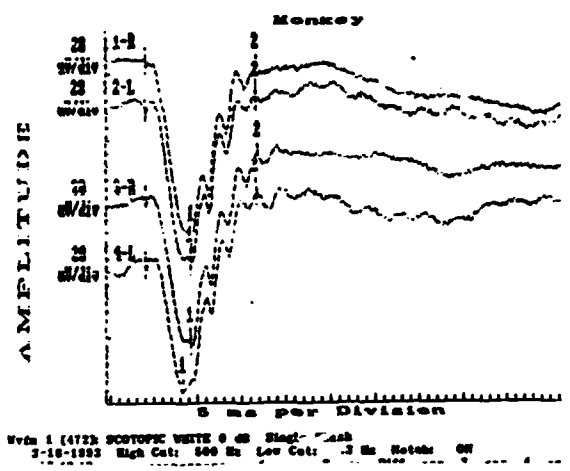
A. (Mainly Rods)



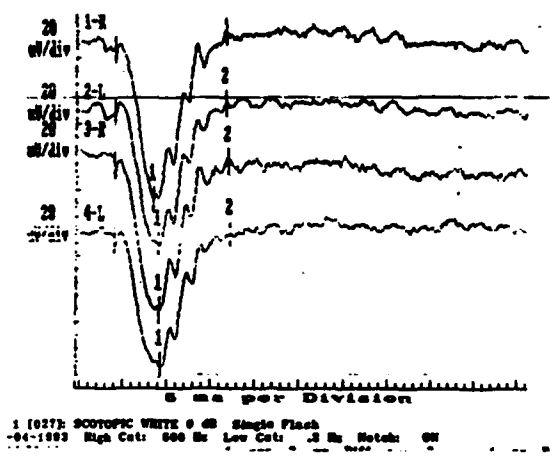
10/2/92 Baseline



11/12/92

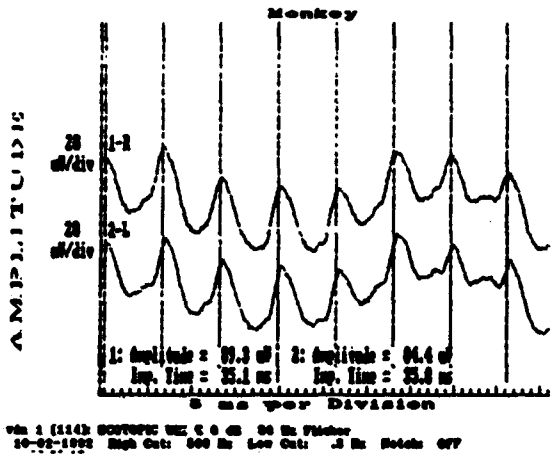


?/16/93

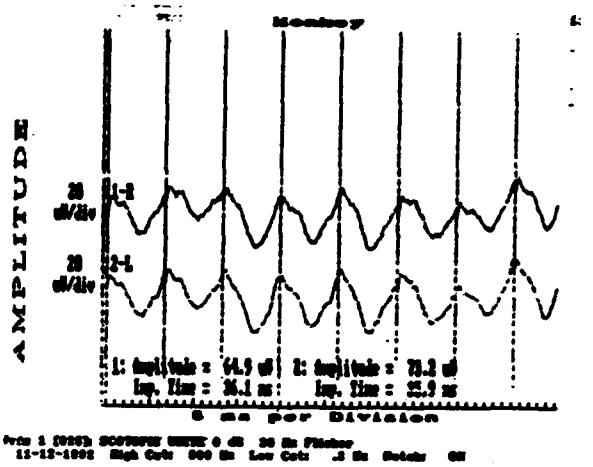


6/4/93

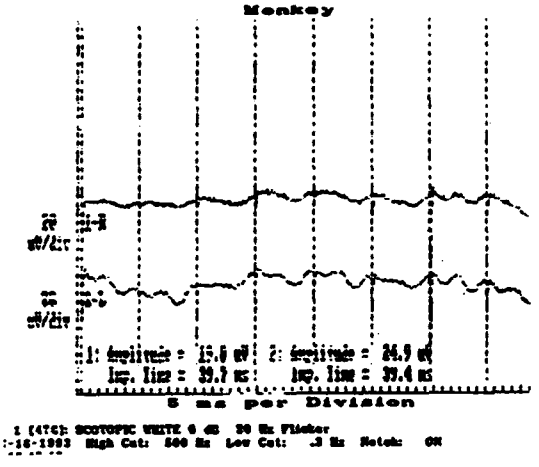
B. (Cones)



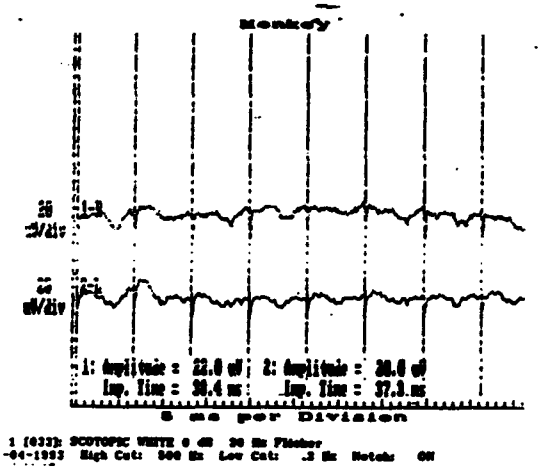
10/2/92 Baseline



11/12/92



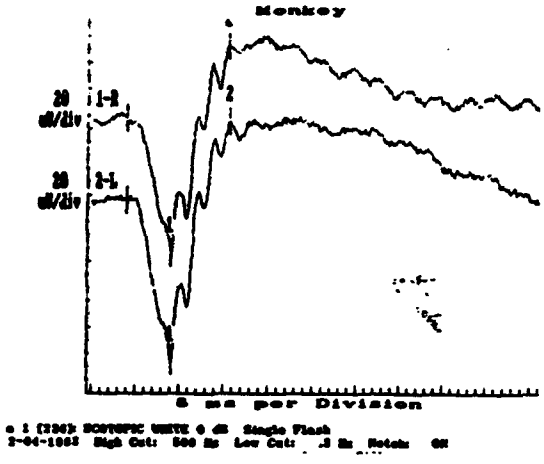
3/16/93



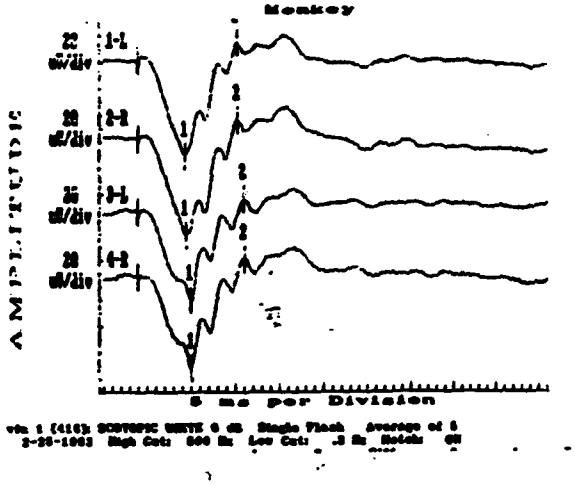
6/4/93

FIGURE 3: ERG RESPONSES FROM MONKEY 11F.

A.

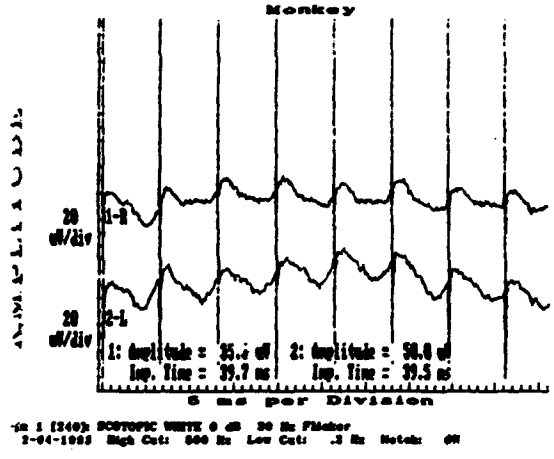


2/4/93 Baseline

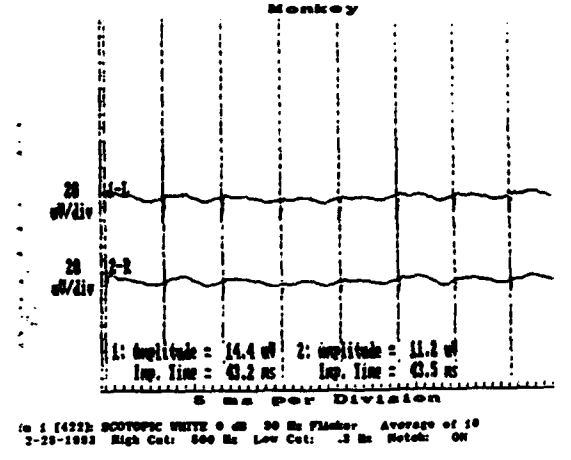


2/25/93

B.



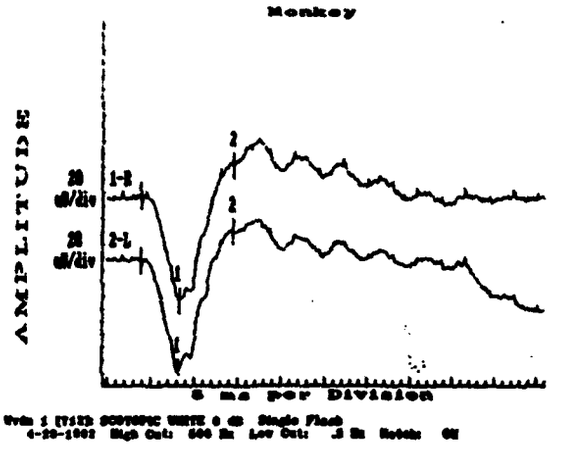
2/4/93 Baseline



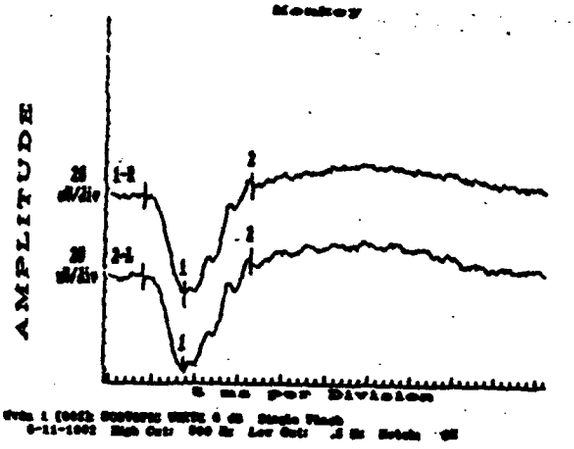
2/25/93

FIGURE 4: ERG RESPONSES FROM MONKEY 21-130.

A.

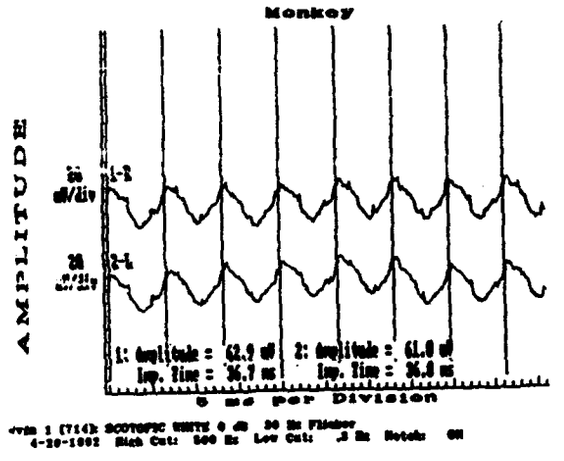


4/29/92 Baseline

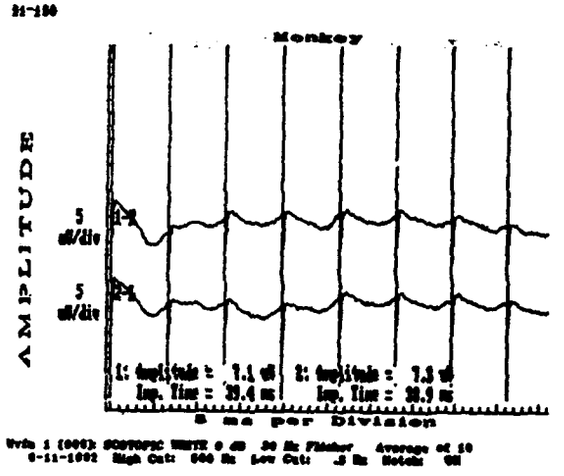


6/11/92

B.



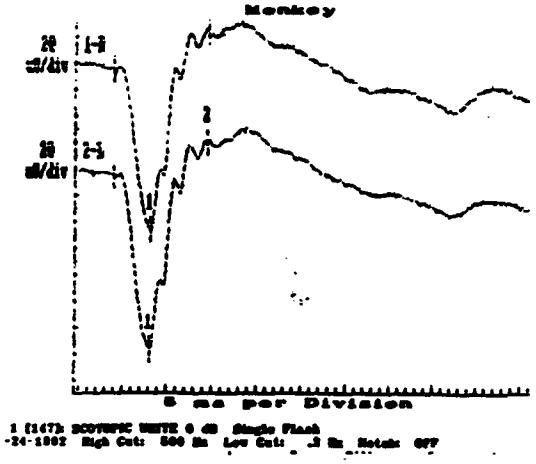
4/29/92 Baseline



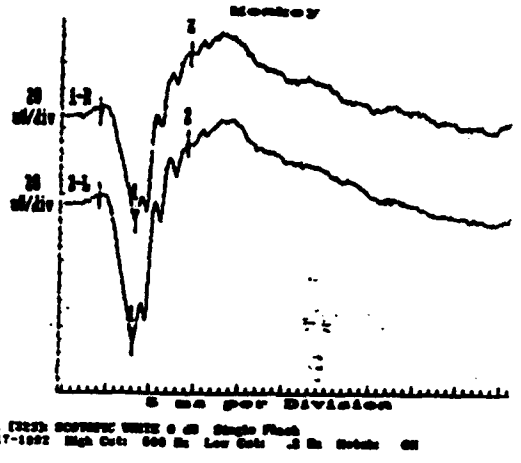
6/11/92

FIGURE 5: ERG RESPONSES FROM MONKEY 82-121.

A.

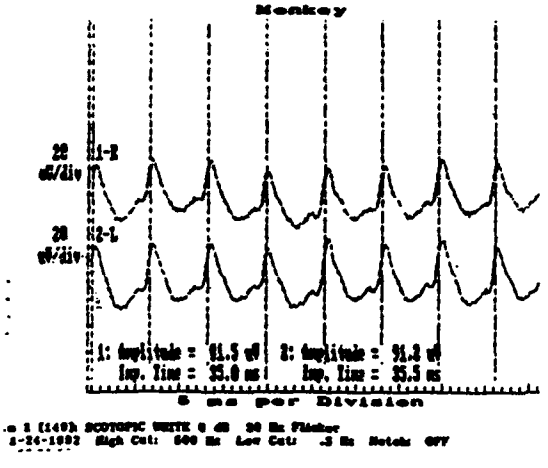


11/24/92 Baseline

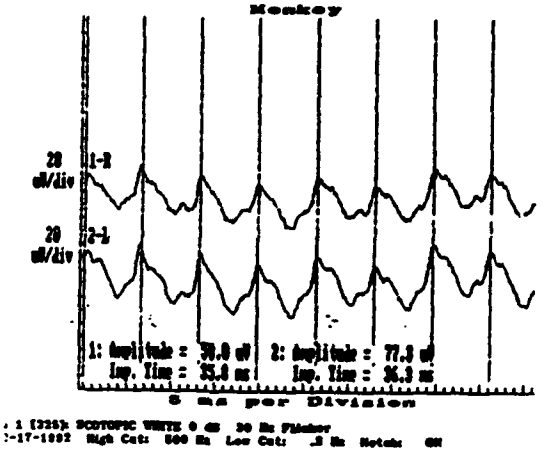


12/17/92

B.



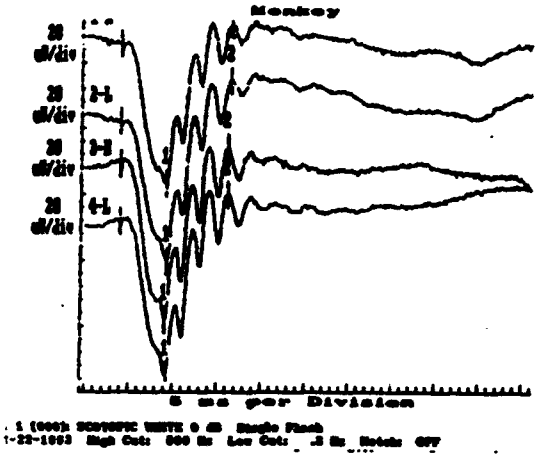
11/24/92 Baseline



12/17/92

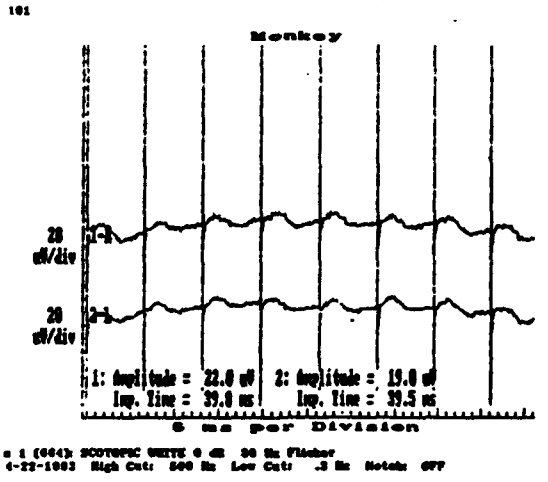
FIGURE 6: ERG RESPONSES FROM MONKEY 82-101.

A.



Post-Exposure 4/22/93

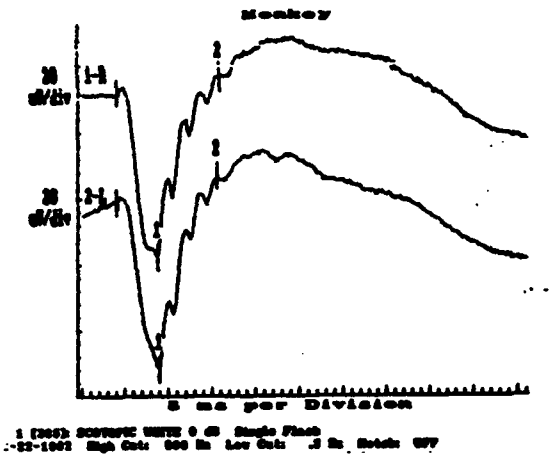
B.



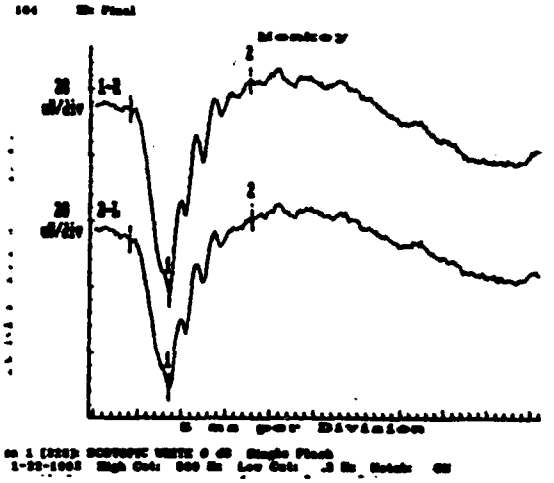
Post-Exposure 4/22/93

FIGURE 7: ERG RESPONSES FROM MONKEY 82-104.

A.

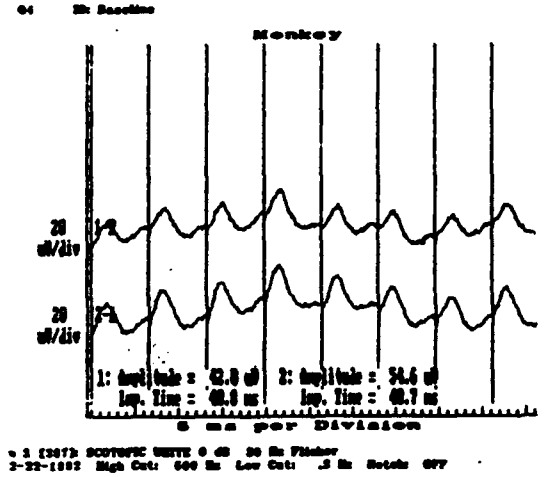


12/22/92 Baseline

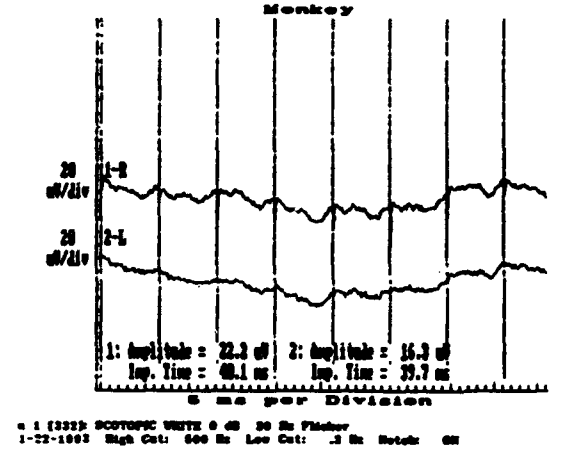


1/22/93

B.



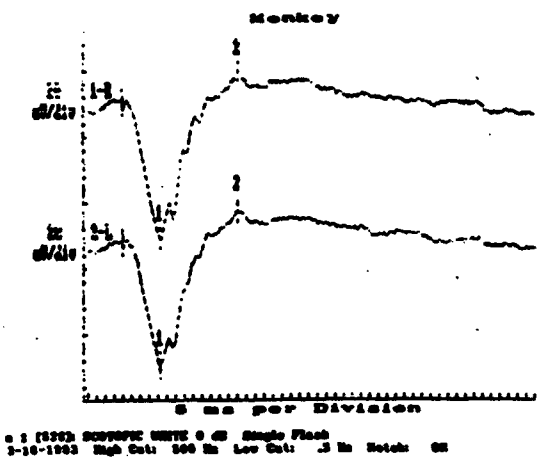
12/22/92 Baseline



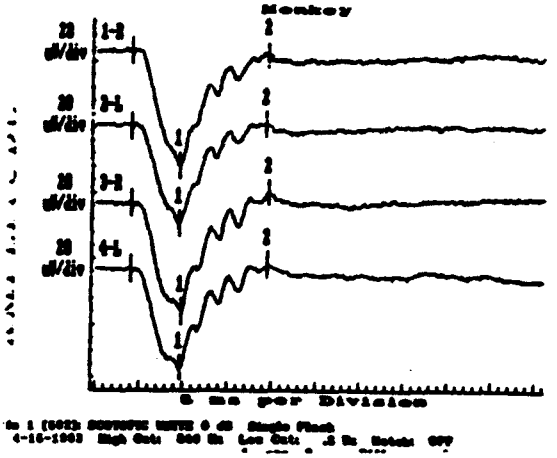
1/22/93

FIGURE 8: ERG RESPONSES FROM MONKEY 82-105.

A.

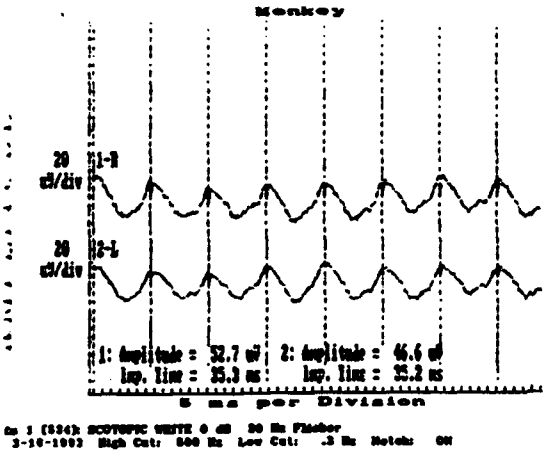


3/16/93 Baseline

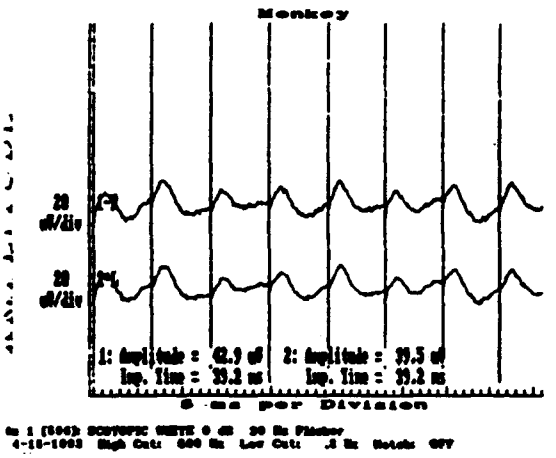


4/15/93

B.



3/16/93 Baseline



4/15/93

ATTACHMENT E

Histopathology Analysis

Performed by

J. Hsu, M.D. and W. Richard Green, M.D.

Wilmer Ophthalmologic Institute

Johns Hopkins University

EYE PATHOLOGY LABORATORY
JOHNS HOPKINS HOSPITAL

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E.P. 94112
E.M. 11610
MONKEY II F
Received: 3/11/93

CLINICAL HISTORY: This juvenile female rhesus monkey underwent a protocol of 3 consecutive weeks of microwave irradiation at Walter Reed Brookville extension.

GROSS: OD: The globe measured 20 x 20 x 19 mm with 5 mm of optic nerve attached. The clear cornea measured 11 x 11 mm. The brown iris enclosed a 7 mm, round pupil. Transillumination was unremarkable. The anterior segment was removed by a circumferential incision 3 mm posterior to the limbus. Intraocular examination was unremarkable.

OS: The globe measured 20 x 20 x 20 mm with 2 mm of optic nerve attached. The clear cornea measured 11 x 11 mm. The brown iris enclosed a 7 mm, round pupil. Transillumination was unremarkable. The anterior segment was removed by a circumferential incision 3 mm posterior to the limbus. Intraocular examination was unremarkable.

From both globes, fragments of the lens, labeled "A" (OD) and "D" (OS), the anterior segment, labeled "B" (OD) and "E" (OS), and the posterior segment containing the macula and optic nerve, labeled "C" (OD) and "F" (OS), were submitted for light microscopy.

Similar fragments of the lens, anterior segment, and posterior segment from the left globe, labeled "A", "B", and "C", respectively, were submitted for TEM.

The remaining fragments were kept in glut-form fixative for storage.

MICROSCOPIC: OD: A: Examination of the lens discloses no abnormalities.

B: Examination reveals an anterior segment with normal cornea. The trabeculum, Schlemm's canal, iris, and ciliary body on both sides are unremarkable.

C: Examination of serial sections of a portion of the posterior segment reveals retina, choroid, sclera, optic nerve, and inferior oblique muscle. The retina has normal lamellar architecture. However, some of the photo-receptors appear slanted and some spaces are present between the photoreceptors and RPE.

OS: D: Examination of the lens reveals no evidence of cataractous change.

E: Examination reveals an anterior segment with normal cornea. The trabeculum, Schlemm's canal, iris, and ciliary body on both sides are unremarkable.

F: Examination reveals a portion of the posterior pole.

The retina has normal lamellar architecture. Some of the photoreceptors appear slightly slanted and some spaces are present between the photoreceptors and RPE.

TEM MICROSCOPIC: Examination of 1-micron thick plastic embedded sections of the left eye reveals the following:

A: Lens with no cataractous changes.

B: Cornea with intact epithelium and Bowman's layer. The stroma measures 0.7 mm and is unremarkable. The endothelium appears swollen with numerous vacuoles.

C: Posterior segment with retina, choroid, sclera, optic nerve and striated muscles.

MICROSCOPIC TEM:

A: Lens (TEM pending)

B: Examination of 13 TEM micrographs (49078-49090) of the left cornea reveals an endothelium with numerous intra- and intercellular vacuoles. Descemet's membrane is unremarkable. The epithelium, Bowman's layer, and anterior stroma are unremarkable.

C: Examination of 17 TEM micrographs of the posterior segment of the macular area of the left eye (49061-49077) reveals markedly distended RPE that measures up to 25 microns in thickness. The basal infoldings and the plasma membrane of the RPE are loss. The cytoplasm has a ground-glass appearance with loss of much of the organelles except for occasional swollen mitochondria. Much of the melanin granules has loss its lancet-shape and appears rounded up. The plasma membrane between RPE cells is loss. There is extensive loss of the apical villous processes and destruction of the plasma membrane of the RPE.

The outer segments of the photoreceptors are irregular in arrangement, disrupted, and swollen. The swelling of some outer segments created large spaces between the cell membrane and distorted lamellar discs. Numerous vacuoles contain disrupted discs and smudged degenerated fragments of outer segments. Many outer segments appear detached from the inner segments where the plasma membrane is disrupted. In some areas, the outer portion of the inner segments appear swollen. There is moderate swelling of mitochondria of the inner segments. The external limiting membrane is unremarkable. In the outer portion of the outer nuclear layer, the nuclei maybe swollen. The neurons in the outer plexiform layer are markedly swollen and contain numerous vacuoles. Cells in the inner nuclear layer appear swollen with marked vacuolization of the cytoplasm around the nucleus, extensive loss of cytoplasmic organelles, and disruption of the plasma membranes. Neurons of the inner plexiform layer are markedly swollen and contain numerous vacuoles. Some ganglion cells are markedly swollen with loss of cytoplasmic organelle and disruption of plasma membrane. The nerve fiber layer has

occasional vacuoles. The internal limiting membrane is continuous.

J. Hsu, M.D.

IMPRESSION:

Animal eyes: Monkey microwave effect on RPE with swelling, loss of apical villous processes, plasma membrane, and basal infoldings, cytoplasmic vacuoles, diminished number of mitochondria
- Outer segments with disruption of plasma membrane and disc, cytoplasmic spaces, electron dense particles, vacuoles
- Inner segments with loss of plasma membrane
- Outer nuclear layer with cytoplasmic vacuoles
- Outer plexiform layer with swollen neurons and neuronal vacuoles
- Inner nuclear layer with extensive cytoplasmic vacuolization, loss of cytoplasmic organelles, and plasma membrane disruption
- Inner plexiform layer with swollen neurons
- Ganglion cells with cytoplasmic organelle loss, vacuoles, plasma membrane disruption, total cellular disintegration in some cells
- Nerve fiber layer w/ vacuoles

Cornea: Endothelium w/ intra- and intercellular vacuoles

EYE PATHOLOGY DIAGNOSIS & CODING:

Experimental studies: Microwave damage (Kues)

W. Richard Green, MD

04/28/93
mc

EYE PATHOLOGY LABORATORY
JOHNS HOPKINS HOSPITAL

Dr. Henry Kues
Applied Physics Lab.
2-211
John Hopkins Road
Laurel, MD 20723

E.P. 94112
E.M. 11610
MONKEY II F
Received: 3/11/93

ADDENDUM

TEM: A: Examination of 3 TEM micrographs reveals a normal lens capsule, epithelium, and cortex.

IMPRESSION:

Animal eyes: Monkey with microwave affect
with normal lens

EYE PATHOLOGY DIAGNOSIS & CODING:

Experimental studies: Microwave affect (Kues)

W. Richard Green, MD

05/13/93
mc

EYE PATHOLOGY LABORATORY
JOHNS HOPKINS HOSPITAL

Dr. H.A.Kues
E.M. 11481

E.P. 93427
MONKEY #82-121
Age: 2 Male

CLINICAL HISTORY: This 2-year-old male rhesus monkey underwent a protocol of 3 consecutive weeks of microwave irradiation at Walter Reed Brookville extension.

GROSS: OD: The globe measured 17 x 17 x 17 mm with 2 mm optic nerve attached. The clear cornea measured 10 x 10 mm. The brown iris enclosed a round 4 mm pupil. The globe was opened horizontally by removal of the superior cap. Intraocular examination disclosed no abnormality.

OS: The globe measured 17 x 17 x 17 mm with 1 mm of optic nerve attached. The clear cornea measured 10 x 10 mm. The brown iris enclosed a round 4 mm pupil. The globe was opened horizontally by removal of the superior cap. Intraocular examination disclosed no abnormality.

The portion of both globe containing the macula and optic nerve are prepared for TEM. A small portion of posterior pole was processed for LM. The remainder of specimens were cut into small pieces and kept in the fixative for TEM.

MICROSCOPIC: OD: Examination of serial sections of a portion of posterior pole reveals normal lamellar architecture of retina. The choroid and sclera are unremarkable.

OS: Examination reveals a similar findings to right eye.

MICROSCOPIC TEM: A: (Right eye): Examination of 1-micron thick plastic embedded sections reveals a posterior segment of the eye including retina, choroid, sclera and a small fragment of optic nerve. The retina has normal lamellar architecture.

B: (Left eye): Examination of 1-micron thick plastic embedded sections reveals a similar findings to A except the absence of the optic nerve in available sections.

TEM: Examination of 11 TEM micrographs (47789-47799) reveals RPE, which contain a moderate number of mitochondria mostly in the basal portion and a small number of melanin, melano-lipofuscin and lipofuscin granules. Numerous basal infolding are present. There are junctional complex between neighboring cells. The zone of outer segments of photoreceptor layer is obliquely sectioned. Even after taking this into consideration, a mild irregularity of arrangement is present in the zone of outer-segments. In some area, there is a gap between the edge of discs and the cell membranes. Disruption of plasma membrane, accumulation of electron dense material and distortion of lamellar discs of photoreceptor outer segments are present. The inner segments of photoreceptor contain numerous mitochondria. The ELM and outer nuclear layer are unremarkable. In the outer-plexiform layer, there

are several vacuoles in the inter- and intracellular spaces (?artifact). The cells on the inner nuclear layer and ganglion cells have a mild degenerative changes with vacuoles in the cytoplasm (?artifact).

B: Examination of 3 TEM micrographs reveals a similar findings to A.

M. Funata, M.D.

IMPRESSION:

Animal Eyes: Monkey microwave effect with mild disruption of the outer segments

EYE PATHOLOGY DIAGNOSIS & CODING:

Experimental studies: Microwave damage (Kues)

W. Richard Green, MD

02/02/93
mc

EYE PATHOLOGY LABORATORY
JOHNS HOPKINS HOSPITAL

Dr. Henry A. Kues/L. Brooks
Woods 274

E.P. 93801
E.M. 11550
MONKEY #82-104
Age: 1.5 - 2 yrs.
Received:

CLINICAL HISTORY: This 1.5 to 2-year-old male Rhesus monkey underwent a protocol of 3 consecutive weeks of microwave irradiation at Walter Reed Brookville extension.

GROSS: OD: The globe measured 17 x 17 x 17 mm with 5 mm of optic nerve attached. The clear cornea measured 10 x 10 mm. The brown iris enclosed a round 4 mm pupil. The globe was opened horizontally by removal of the superior cap. Intraocular examination disclosed no abnormalities.

OS: The globe measured 17 x 17 x 17 mm with 2 mm of optic nerve attached. The clear cornea measured 10 x 10 mm. The brown iris enclosed a round 4 mm pupil. The globe was opened horizontally by removal of the superior cap. Intraocular examination disclosed no abnormalities. The portion of both globe containing the macula and optic nerve are prepared for TEM. A small portion of posterior pole is processed for LM. The corneal button was bisected and half sent for LM and half sent for TEM.

MICROSCOPIC: OD: A: Examination of serial sections of a portion of posterior pole reveals normal lamellar architecture of retina. The choroid and sclera are unremarkable.

C: Examination reveals a corneal button with intact epithelium and Bowman's layer. The stroma measures 0.6 mm centrally. Descemet's membrane is intact. There are 20 endothelial cell nuclei/HPF. The trabeculum and Schlemm's canal are present near one margin.

OS: B: Examination of serial sections of a portion of posterior pole reveals normal lamellar architecture of retina. The choroid and sclera are unremarkable.

D: Examination reveals a corneal button with intact epithelium and Bowman's layer. The stroma measures 0.6 mm centrally. Descemet's membrane is intact. There are 19 endothelial cell nuclei/HPF. The trabeculum is present on both margin.

MICROSCOPIC TEM: A: Examination of 1-micron thick plastic embedded sections of the right eye reveals a posterior segment with retina, choroid, sclera, optic nerve in cross-sections, and striate muscle in cross-section. The retina has normal lamellar architecture. There is some slanting of the photoreceptor and some spaces are present between the photoreceptors and the RPE.

B: Examination of 1-micron thick plastic embedded sections of the left eye reveals a posterior segment with similar findings to the right eye except for the absence of the optic nerve in available sections. Many of the photoreceptors are cut in cross-section. Some microcystic changes are present between the photoreceptors and the RPE.

C: Examination of 1-micron thick plastic embedded sections of the right eye reveals a corneal button with intact epithelium and Bowman's layer. The stroma measures 0.6 mm centrally and is unremarkable. The endothelium appears swollen with numerous vacuoles.

D: Examination of 1-micron thick plastic embedded sections of the left eye reveals a corneal button with similar findings as in the right eye (C).

TEM: A: Examination of 12 TEM micrographs (48487-48498) of the right posterior segment reveals RPE with a moderate number of mitochondria basally and a small number of melanin, melanolipofuscin, lipofuscin granules. There are tight junctional complexes between neighboring RPE cells. The RPE appears distended with a ground-glass appearance to much of the cytoplasm. There is loss of much of the apical villous processes and the plasma membrane in some areas. The basal infoldings of the RPE are ill-defined. The plasma membrane between RPE cells is loss in areas. The outer segments of photoreceptors displays moderate irregularly in arrangement. Disruption of the outer segment plasma membrane and discs causing lucent gaps between the edge of the discs and the cell membrane, the accumulation of vacuoles measuring up to 1-micron in diameter which contain fragments of disrupted discs and degenerated particles are present. Many outer segments appear detached from the inner segments where the plasma membrane appear disrupted. The external limiting membrane is unremarkable. In the 1 to 2 outer layers of the outer nuclear layer, the nuclei are swollen with dispersion of chromatin. In some areas, the plasma membrane of the inner segments is lost. The neurons in the outer plexiform layer are markedly swollen and contain numerous vacuoles. The cells in the outer aspect of the inner nuclei layer are swollen, contain many cytoplasmic vacuoles and have loss of cytoplasmic organelles. The inner aspect of the inner plexiform layer, the ganglion cell layer, the outer plexiform layer, the nerve fiber layer and the internal limiting membrane are not present in the available TEM micrographs.

B: Examination of 9 TEM micrographs (48499-48507) of the left posterior segment reveals similar, somewhat less intense findings to A.

C: Examination of 8 TEM micrographs (48517-48523) of the right cornea reveals numerous intra- and intercellular vacuoles in the endothelium. Occasional tight junctions are present between neighboring cells. The mitochondria are slightly swollen and have ill-defined cristae. Descemet's membrane, posterior stroma, anterior stroma, Bowman's layer, and the epithelium are unremarkable.

D: Examination of 8 TEM micrographs (48508-48515) of the left cornea reveals similar findings to C.

M.Funata/J.Hsu, M.D.

IMPRESSION:

Animal eyes: Monkey microwave effect w/ RPE w/ swelling, loss of apical villous processes and plasma membrane, ill-defined basal infoldings;
Outer segments w/ disruption of plasma membrane and discs, lucent cytoplasmic gaps, electron dense particles, vacuoles;
Inner segments w/ loss of plasma membrane;
Outer nuclei layer w/ nuclear swelling and chromatin dispersion;
Outer plexiform layer w/ swollen neurons and neuronal vacuoles;
and Inner nucleus layer w/ swollen cells w/ cytoplasmic vacuoles, loss of cytoplasmic organelles

Cornea: Endothelium w/ intra- and intercellular vacuoles, diminished tight junctions, and swollen mitochondria

EYE PATHOLOGY DIAGNOSIS & CODING:

Experimental studies: Microwave damage (Kues)

W. Richard Green, MD

03/18/93
mc

EYE PATHOLOGY LABORATORY
JOHNS HOPKINS HOSPITAL

Dr. Henry A. Kues, Jr.
Woods 274

E.P. 94484
RHESUS MONKEY 82-105
Received: 4/21/93

CLINICAL HISTORY: This juvenile rhesus monkey underwent a protocol of 3 consecutive weeks of microwave irradiation at Walter Reed Brookville extension.

GROSS: OD: The globe measured 17 x 17 x 18 mm with 3 mm of optic nerve attached. The clear cornea measured 9 x 10 mm. The brown iris enclosed a 6 mm pupil. Transillumination was unremarkable. The globe was opened by removal of the superior cap. Intraocular examination was unremarkable. A strip of the posterior segment labeled "A", a sector of the lens labeled "B", and a strip of the cornea labeled "C" was submitted for electron microscopy. The remaining ocular structures were stored in glutform.

OS: The globe measured 17 x 17 x 17 mm with 2 mm of optic nerve attached. The clear cornea measured 11 x 10 mm. The brown iris enclosed a 7 mm pupil. Transillumination was unremarkable. The globe was opened by removal of the superior cap. Intraocular examination was unremarkable. The pupil-optic nerve (PO) segment was submitted for light microscopy. The remaining ocular structures were stored in glutform.

MICROSCOPIC: Examination discloses a normal cornea with 20 endothelial nuclei/HPF. The angle, iris, ciliary body, and vitreous are unremarkable. Step sections through the macula reveals slanting and mild disruption of the photoreceptor outer segments. Numerous vacuoles are present between the photoreceptors segments and RPE. The remainder of the retina are unremarkable. The RPE appears normal. The choroid and sclera are unremarkable. Longitudinal and cross-sections of the optic nerve are unremarkable.

J. Hsu, M.D.

EYE PATHOLOGY DIAGNOSIS & CODING:

Experimental studies: Microwave exposure (Kues)

W. Richard Green, MD

06/23/93
mc

EYE PATHOLOGY LABORATORY
JOHNS HOPKINS HOSPITAL

Dr. Henry A. Kues
Woods 274

E.P. 94550
MONKEY 82-101
TEM: 11690
Received: 4/22/93

CLINICAL HISTORY: This juvenile rhesus monkey underwent a protocol of 3 consecutive weeks of microwave irradiation at Walter Reed Brookville extension.

GROSS: OD: The globe measured 19 x 18 x 19 mm with 1 mm of optic nerve attached. The clear cornea measured 11 x 10 mm. The brown iris enclosed an 8 mm pupil. Transillumination was unremarkable. The globe was opened by removal of the superior cap. Intraocular examination was unremarkable. A strip of the posterior segment labeled "A", a sector of the lens labeled "B", and a strip of the cornea labeled "C" was submitted for electron microscopy. The remaining ocular structures were stored in glutform.

OS: The globe measured 19 x 19 x 18 mm with 2 mm of optic nerve attached. The clear cornea measured 11 x 10 mm. The brown iris enclosed an 8 mm pupil. Transillumination was unremarkable. The globe was opened by removal of the superior cap. Intraocular examination was unremarkable. The pupil optic nerve (PO) segment was submitted for light microscopy. The remaining ocular structures were stored in glutform.

MICROSCOPIC: OS: Examination reveals a normal cornea with 18 endothelial nuclei/HPF. The angle, iris, ciliary body, lens, and vitreous are unremarkable. The retina has normal lamellae architecture. Step sections through the macula disclose minimal abnormalities. Some of the photoreceptors appear slightly slanted and some spaces are present between the photoreceptors and RPE (?artifact). The choroid, sclera, and optic nerve are unremarkable.

MICROSCOPIC TEM: OD: A-Examination of 1-micron thick plastic embedded sections reveals a posterior segment including the macular, choroid, sclera, and a small segment of the optic nerve. The macula has normal lamellar architecture. The nerve fiber layer and the inner plexiform layer has a dense vesicular appearance. The cells of the ganglion cell layer and the inner nuclear layer have prominent perinuclear halos. The outer plexiform layer has some larger vacuoles of its innermost portion. The outer nuclear layer and the photoreceptor inner segments are minimally disrupted. The photoreceptor outer segments are moderately slanted and some spaces are present between the photoreceptors and RPE. The RPE is unremarkable. The choroid, sclera, and optic nerve are unremarkable.

B: Examination of 1-micron thick plastic embedded section discloses a small wedge of lens compose of normal capsule, epithelium and cortex.

C: Examination of 1-micron thick plastic embedded section discloses a cornea with an intact epithelium and Bowman's layer. The stroma is unremarkable and measures 0.6 mm. Descemet's membrane is unremarkable. Endothelium appears normal in the most part. There is an abrupt transition where the endothelium is markedly vacuolated.

TEM: A: Examination of 13 TEM micrographs (49907-49919) reveals RPE with a moderate number of mitochondria mostly in the basal portion and a small number of melanin, melanolipofuscin, and lipofuscin granules. The basal infoldings and the plasma membrane between RPE cells are ill-defined. The apical villous processes are mild disrupted with vesicular formation. The outer segments of the photoreceptors display moderate irregularity in arrangement. There is disruption of the outer segment plasma membrane and discs causing lucent gaps to form between the edge of the discs and the cell membrane. Numerous vacuoles measuring up to 5 microns in diameter containing disrupted discs particles are present. The external limiting membrane is unremarkable. In the outer layers of the outer nuclear layer, the nuclei are swollen with dispersion of chromatin. The outer most extent of the outer plexiform layer contains large vacuoles measuring upto 10 microns in diameter. The cells in the outer nuclear layer and the ganglion layer are swollen, contains numerous cytoplasmic vacuoles, and have loss of cytoplasmic organelles. Some disruption of the nuclear and plasma membranes are present. The nerve fiber layer is markedly disrupted with numerous cystic spaces measuring 2-6 microns in diameter. The ILM is unremarkable.

C: Examination of 14 TEM micrographs (49928-49941) discloses a normal corneal epithelium and Bowman's layer. In one area, the endothelium appears normal. Endothelium is slightly thickened and has a numerous intracytoplasmic vacuoles measuring 1.8 x 2 microns. Descemet's membrane and posterior stroma are unremarkable. The keratocytes appear normal.

J. Hsu, M.D.

IMPRESSION:

Animal eyes: Monkey microwave effect w/ RPE w/ ill-defined plasma membrane and basal infoldings, diminished number of granules, loss of apical processes with vesicle formation;
Outer segments w/ disruption of plasma membrane and discs, lucent cytoplasmic gaps, electron dense particles, vacuoles;
Outer nuclear layer w/ nucleus swelling and chromatin dispersion;
Outer plexiform layer w/ vacuoles;
Inner nuclear layer & ganglion cell layer with swollen cells w/ cytoplasmic vacuoles, loss of organelles, disruption of nuclear & plasma membrane;
Nerve fiber layer w/ marked disruption of axons with vacuole formation
Cornea: Endothelium w/ intra- and intercellular vacuoles

EYE PATHOLOGY DIAGNOSIS & CODING:

Experimental studies: Microwave damage (Kues)

W. Richard Green, MD

07/29/93
mc

EYE PATHOLOGY LABORATORY
JOHNS HOPKINS HOSPITAL

Dr. Henry A. Kues, Jr.
Woods 274

E.P. 94827
MONKEY 82-125
Received: 6/3/93

CLINICAL HISTORY: This juvenile female rhesus monkey underwent a protocol of 3 consecutive weeks of microwave irradiation at Walter Reed Brookville extension.

GROSS: OD: The globe measured 19 x 19 x 18 mm with 5 mm of optic nerve attached. The clear cornea measures 11 x 10 mm. The brown iris enclosed a 7 mm pupil. Transillumination was unremarkable. The globe was opened vertically. Intraocular examination was unremarkable.

OS: The globe measured 19 x 19 x 18 mm with 1 mm of optic nerve attached. The clear cornea measured 11 x 10 mm. The brown iris enclosed a 6 mm pupil. Transillumination was unremarkable. The globe was opened vertically. Intraocular examination was unremarkable. PO sections from both eyes were submitted for light microscopy.

MICROSCOPIC: OD: Examination discloses a normal cornea with 18 endothelial nuclei/HPF. The angle, iris, ciliary body, and vitreous are unremarkable. Step section to and serial sections through the macula reveals slanting and mild disruption of the photoreceptor inner and outer layers. Mild vacuolization of the photoreceptor outer segments is present (slide 26, foveola). The RPE appears normal. Longitudinal sections of the optic nerve head are unremarkable.

OS: Examination with step sections to and serial sections through the fovea reveals similar findings (slide 50 g foveola).

J. Hsu, M.D.

EYE PATHOLOGY DIAGNOSIS & CODING:

Experimental studies: Microwave exposure (Kues)

W. Richard Green, MD

07/15/93
mc

Within normal range...

ATTACHMENT F

WHITE PAPER

"Enhanced and/or Diminished Effectiveness of Biological and Chemical Agents, Pharmacological Countermeasures, and Biological Sensor Systems in Complex Electromagnetic Environments"

Henry A. Kues
The Johns Hopkins University
Applied Physics Laboratory
Laurel, MD 20723

July 15, 1993

Background

Over the last 30 years, the interaction of electromagnetic environments with biological systems has been investigated extensively. Most of the work has been associated with health hazard assessment; minimal effort has been directed at studies that address mechanisms of interaction responsible for reported biological effects other than classic (or bulk) thermal heating (and photochemical effects at shorter wavelengths). The literature provides increasing evidence that complex electromagnetic environments, such as those encountered under battlefield conditions, could directly affect certain military systems and troop performance. The following paragraphs identify some specific areas of military concern that should be investigated.

Our investigations and those of others have repeatedly shown that exposure of experimental subjects to electromagnetic environments can cause breakdown of vascular barriers, including those in the brain and eye, and can influence drug activity and/or neural transmission[1-6]. For example, we have reported electromagnetically induced effects, such as corneal endothelial lesions, increased permeability of the iris vasculature, altered retinal electrophysiologic activity (visual function), and histopathological changes[7]. The administration of specific drugs, including beta blockers, can significantly enhance these effects, as evidenced by a lowered damage threshold exposure level[8]. The electromagnetic environment can also alter the drug's action, as has been demonstrated with the anticholinesterase drug, physostigmine[9]. Other investigators have shown an alteration of the brain's opioid system following exposure to an electromagnetic environment[10]. Also of interest is the reported increased virulence of Japanese Encephalitis Virus under the influence of an electromagnetic environment[11]. Whether this latter effect is related to disruption of the blood/brain barrier remains to be explored.

Research Recommendations

The effects reported lead to the conclusion that complex electromagnetic environments, such as those produced under battlefield conditions, can alter the effectiveness of biological and chemical warfare agents and pharmacological countermeasures to these agents. Research should be conducted

[illegible]

to define either the enhanced or diminished effects of such biological and chemical warfare agents and the countermeasures to them. Also, after reviewing the accessible literature, it is possible to theorize that complex electromagnetic environments can impact biological sensor systems that are currently under development. This theory should be investigated to determine whether the sensitivity of these systems is altered by exposing them to these environments.

In considering the previously described agents their and countermeasures such as physostigmine and/or its analogues, reportedly used during Operation Desert Storm, studies to determine the ability of complex electromagnetic environments to alter these compounds and their actions on troops should be undertaken. An initial effort should focus on whether the mechanism of the electromagnetically induced effect is based on changes in absorption, metabolism, or direct compound alteration.

In parallel with the development of any biological sensor system, a study should be undertaken to determine whether complex electromagnetic environments can adversely affect either the biological sensors or the sensitive electronic components within the detector system, thus hindering the system's capabilities. The results of such a project could then be incorporated into design specifications during development of a prototype that will address sensitivities to complex electromagnetic environments before field deployment.

Related Research Areas

Certain technologies under development for use both in the medical community and in advanced military weapons systems are related to complex electromagnetic/biological sensor effects. A technology is being developed to use biological interfaces to control the movement of electromechanical devices, with applications in medicine and in advanced weapons systems. The medical application of direct neurosensory control has focused on precise regulation of prosthesis movement, whereas, the military application is focusing on advanced weapons control. The complex electromagnetic/biological sensor effects involved in this technology make it imperative to investigate the potential for electromagnetic environments to adversely impact its operation.

Studies that address the relationship between complex electromagnetic fields, the environment, and troop performance should also be considered. Studies in this area might include examination of the interaction between electromagnetic environments and toxic compounds (i.e., hydrocarbons encountered in the smoke from oil well fires).

Since other chemical compounds (pesticides and insecticides) are frequently found in areas of troop deployment, questions arise as to electromagnetic environmental interaction with these compounds and possible synergistic effects on toxic damage thresholds. Investigate of electromagnetic effects on these compounds and the possible consequent changes in susceptibility to parasitic, bacterial, and viral infection is warranted.

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ATTACHMENT G

TO : PHONE NO. : 12106588022 MAY. 24. 1993 7:09PM P 1
FROM : ACCESS INT'L PRESS, INC. (Printing & Copying Center PHONE NO. : 301 270 8804

A RESEARCH GUIDE FOR DESERT STORM SYNDROME

By Patricia Axelrod
308 Boyd Ave., Takoma Park, Md.
301-270-8622

Conducted at the request of Staff Sergeant Carol Picou
San Antonio, Texas, 210-658-7870
and
Ms. Rosemary Torres, National Institutes of Health,
Office of Women's Health Research

With contributing material by Staff Sergeant Carol Picou; Dr. Thomas J. Callender, Med-Health, Ltd. Lafayette, La.; Dr. Barry Wilson, Battelle, Pacific Northwest Laboratories, Richland, Washington, and Commissioner Rudy Arredondo, Maryland Governor's Commission on Black and Minority Health.

Submitted May 10, 1993

DRAFT

① Cover Sheet Fax to C. Picou
210. 658-8022
② Carol's Fax to T. Callender
Dear Lady Picasso:
" " Barry Wilson at Battelle

Patricia Axelrod
308 Boyd Ave.
Takoma Park, Md. 20912
301-270-8622

May 7, 1993

Ms. Rosemary Torres, Director
The Office of Research on Women's Health
Building 1, Room 201
National Institutes of Health-ORWH
9000 Rockville Pike
Bethesda, MD 20892
301-402-1798 fax, 301-402-1770 phone

Dear Ms. Torres:

Pursuant to our recent phone conversations, I am pleased that the National Institutes of Health (NIH) have chosen to undertake a preliminary review of what has come to be known as "Desert Storm Syndrome." I seek your assistance on behalf of U.S. Army Staff Sergeant Carol Picou, San Antonio, Texas and the thousands of other Desert Storm soldiers, who like her, have been struck by this mysterious illness following their service in the Persian Gulf War. Sergeant Picou has offered her partial medical records for your consideration as typical of Desert Storm Syndrome (available upon request.)

To facilitate your effort, as per your request, I am presenting a consolidation of my initial investigation into this matter. The purpose of this document is to function solely as a guideline to future research by interested parties. Staff Sgt. Picou and her attending civilian physician, Dr. Thomas Calander, contributed to this report along with Dr. Barry Wilson, Batelle Pacific Northwest Laboratories and Commissioner Rudy Arredondo, Maryland Governor's Commission on Black and Minority Health.

But first a word about my qualifications and interest in Desert Storm Syndrome. I am a scientific writer and researcher and a recent recipient of a $60,000.00 John D. and Catherine T. MacArthur Foundation (Chicago, Illinois) Research and Writing grant for my work on the effects of electromagnetic radiation. I am a past research associate of Dr. Paul Smoker, Lancaster University - Lancaster, England and in 1987 I was awarded a "Project Censored" award by Sonoma University, School of Media Studies, Sonoma, California. This summer, I am a visiting professor at the Johns Hopkins University, School of Arts and Sciences, (Washington, D.C. campus) where I am teaching graduate level investigative research techniques and methodology.

My MacArthur Grant was awarded in November 1990, just prior to Desert Storm. I decided to invest the award in an analysis of the war. In the course of this work, I investigated friendly fire deaths and my efforts resulted in a General Accounting Office (GAO) investigation into the friendly fire killing of Corporal Lance Fielder.

Desert Storm Syndrome first came to my attention when I read several articles detailing numerous undiagnosed ailments affecting Desert Storm soldiers. Later, I personally interviewed soldiers who confirmed those symptoms described by the popular press. The chronic symptoms include shortness of breath, loss of balance and fatigue, flu-like chills and sweats, diarrhea and loss of bladder control, headache and head pressure, joint and muscle pain, chest pain and soreness, sores, skin rashes and burning skin, nose bleeds, bleeding gums and loosened teeth, hoarseness, hair loss, water retention, abdominal pain and bloating, seizures, confusion, sleeplessness, blurred vision and short term memory loss. These symptoms are often accompanied by graying hair, ridging and splitting fingernails and uncontrolled weight loss or gain. A disproportionate number of young women soldiers report gynecological problems, resulting in hysterectomy. Men report impotence. In addition, there are numerous reports of miscarriages as well as stillborn, underweight and malformed babies born to Desert Storm families.

An initial review of available scientific literature suggests that there is sufficient reason to believe that any one of a number of factors - ranging from the effects of depleted uranium to pesticide exposure - may be causing the soldiers to fall ill. Unfortunately, the Department of Defense (DOD) regards Desert Storm Syndrome in much the same way as Agent Orange was initially viewed. Rather than seriously investigating and diagnosing the soldier's ailments, the DOD has chosen to ignore and discount their chronic sickness; often mitigating the symptoms by categorizing them as Post Traumatic Stress Disorder (PTSD). In short, the complaining soldier is told "it's all in your head" and then summarily dismissed by military physicians who order mental evaluations, muscle relaxants and sleeping pills. It is for this reason that your help is so vital. Surely, if the tragedy of Agent Orange taught us anything, it was NOT to ignore a soldier's symptoms and very real suffering.

The following is an informal consolidation of facts and medical findings pertinent to Desert Storm Syndrome. It is presented as a guideline to future research needs only, and unless otherwise stated, does not include source documentation. The information contained was primarily obtained by personal interviews with experts and from The National Technical Institute of Standards (NTIS) data base. The World Health Organization (WHO) provided most of the information on leishmaniases. More detailed references are available upon request. Please be advised that the National Institutes of Health have either in the past, or are now currently funding studies which should be helpful to understanding Desert Storm Syndrome.

On behalf of Staff Sergeant Picou and countless others I thank you in advance for your cooperation.

I. Desert Storm Syndrome symptoms:

The chronic symptoms include shortness of breath, loss of balance and fatigue, flu-like chills and sweats, diarrhea and loss of bladder control, headache and head pressure, joint and muscle pain, sores, skin rashes and burning skin, bleeding gums and loosened teeth, nose bleeds, hoarseness, hair loss, water retention, abdominal pain and bloating, chest pain and soreness, confusion, seizures, sleeplessness, blurred vision and short term memory loss. These symptoms are often accompanied by graying hair, ridging and splitting fingernails and uncontrolled weight loss or

gain. A disproportionate number of young women soldiers report gynecological problems, resulting in hysterectomy. Men report impotence. In addition, there are numerous reports of miscarriages as well as stillborn, underweight and malformed babies born to Desert Storm families.

II. Preliminary research defines the following potential causes for Desert Storm Syndrome:

1. Administration of three vaccines intended as protection against nerve and biological warfare agents.

A. Pyridostigmine - an unproven vaccine intended to protect against nerve warfare.

a. Pyridostigmine has been prescribed since 1955 for myasthenia gravis, a rare auto-immune disease involving the faulty transmission of nerve impulses to the muscles. Myasthenia gravis is a disorder that occurs when the immune system becomes defective and produces antibodies that disrupt the signals being transmitted between the nervous system and the muscles under voluntary control. The result is a progressive weakening of the muscles beginning with the muscles that control the eyes, face pharynx and larynx with a more gradual weakening of the legs and arms. The disease is linked to a disorder of the thymus gland, which is thought to be partly responsible for the abnormal antibody activity.

b. The dosage of pyridostigmine administered daily as treatment for myasthenia gravis varies from 60 to 1500 mgs. daily. Actual doses and administration schedule are ordered to be determined for each patient individually. Therapeutic effects in myasthenia gravis are increased muscle strength and endurance. Pryridostigmine syrup or by injection will permit a finer adjustment of dosage as well as provide for easier absorption with fewer side effects.

c. Pyridostigmine should be taken with food or milk to reduce the intensity of expected side-effects which include small pupils, watering of eyes, slow pulse, excessive salivation and sweating, nausea, vomiting, stomach cramps, diarrhea, and urge to urinate. Mild effects listed as allergic reactions are indicated by skin rash, nervousness, anxiety, unsteadiness, muscle cramps, twitching, and loss of hair. Serious side effects are confusion, slurred speech, seizures, difficult breathing, (asthmatic wheezing) muscle weakness, paralysis and excessive urination, diarrhea and vomiting which may induce low blood potassium levels.

d. Persons exposed to heat are advised to use caution while taking this drug as heat may increase sweating and weakness. e. Exposure to environmental chemicals such as insecticides Baygon, Diazinon and Servin should be avoided as they can accentuate the potential toxicity of this drug. (The extensive use of pesticides and insecticides throughout the Persian Gulf theater heightens the possibility of this synergistic effect.)

f. Pyridostigmine tablets as a vaccine against nerve warfare were administered to Desert Storm soldiers in the amount of 90 mgs. daily taken over 14 to 17 days resulting in a total average intake of 1260 mgs to 1530 mgs. After ingestion, many soldiers immediately complained of side effects, beginning with severe frothing of the mouth. Research indicates frothing as a sign of overdose.

g. Overdosage and pyridostigmine toxicity in a healthy person can lead to a mixed variety of inhibitory and stimulatory responses in the central nervous system which tend to mime myasthenia gravis. (See April 30, 1993 letter from Dr. Barry W. Wilson, Battelle Pacific Northwest Laboratories.) The onset of seizures may suggest epilepsy. In addition, latent

bronchial asthma may be activated.

h. Atropine, when administered early, is effective in blocking many of the adverse effects of pyridostigmine toxicity. (Wilson, April 30, 1993.)

i. There are reports of significant muscular weakness in newborn infants whose mothers have taken this drug during pregnancy. While it is likely that pyridostigmine is not passed to breast milk, nursing mothers are advised to monitor infants closely and discontinue nursing if adverse effects develop in the child.

j. Pyridostigmine substitutes may be equally effective as nerve agent vaccines with fewer side effects . These include Neostigmine, (marketed as Prostigmin) and Normeostigmine and Norpyridestigmine, both of which are currently under consideration as treatment for Alzheimer's disease.

B. Botulinum Pentavalent - another unproven vaccine intended to protect against botulism. There is little information available on this highly experimental vaccine. Research on this vaccine is conducted at the Fort Detrick U.S. Army Medical Research Institute of Infectious Disease. A spokesperson for The Center for Disease Control explains that because of the small number of people who contract botulism there is little opportunity to demonstrate the efficacy of this vaccine. Therefore, the vaccine will remain unproven and unlicensed. It is unclear how many soldiers were vaccinated.

C. Anthrax - to protect against the disease anthrax. There are three forms of this vaccine: 1) a mutant of the veterinary vaccine B. anthacis Sterne strain, 2) recombinant, live which produce protective antigen, 3) non-living vaccines containing protective antigens and new adjuvant formulations. Research on this vaccine is underway at Fort Detrick. The Center for Disease Control, Atlanta, Ga. does distribute a licensed vaccine which they state has minimal short term side effects. Because it has not been tested in pregnant women, some Desert Storm medical personnel warned people taking the vaccine against having a child for 3-4 years after taking the vaccine. The origin of the decision to issue this warning is uncertain. This vaccine was apparently selectively administered.

2. The effects of depleted uranium used in tank armor, missile and aircraft counterweights and navigational devices, and in tank and anti-aircraft and personnel artillery:

a. The most relevant findings regarding the effects of depleted uranium were produced by the Radium Research Project, New Jersey State Department of Health, Trenton, New Jersey in their report entitled "Epidemiological Follow-up of The New Jersey Radium Cases, November 1957 Through 1967" published by the Untied States Atomic Energy Commission, Division of Technical Information. This study was conducted as an effort to help establish safe exposure levels to radioactive materials. As such, the study describes ten years of epidemiological follow up of a group of dial painters and other radium workers exposed more than 45 years earlier to depleted uranium used as the activator for radioluminous watch, clock and instrument dial paint.

b. At the time of the study many of the workers were still alive and carried measurable, but very low, body burdens of radioactive isotopes.

c. The symptoms generally described by the workers and detailed in the "Epidemiological Follow -up..." were reiterated in the singular case history of one of the workers in a report entitled, "Radium Osteitis With Osteogenic Sarcoma: The Chronology and Natural History of

a Fatal Case," by Dr. William D. Sharpe and published in the Bull. N.Y. Acad. Med., Vol. 47, No. 9, September 1971. The subject's symptoms were recorded throughout the early 1920's and into the early 30s by her attending physician. She died in 1933 at about age 31, almost 16 years after her first exposure to the depleted uranium contained in the dial paint. Her medical records were recovered and incorporated into the Atomic Energy Report and also served as the basis for Dr. Sharpe's report. After working as a dial painter for about four years, she first became ill in 1923, two years after the termination of employment. Her initial symptoms included necrosis of the jaw and other jaw infections, loosened teeth and neuralgia-like pains, stomach trouble, pains described by the subject as neuralgia-like throughout her body, mysterious throat infections and nervousness. Later, she developed fluid in the knee. In 1926, her physician reported she had begun to limp and "roetgenograms showed beginning radiation osteitis in the lower end of the femur." She sustained a spontaneous fracture which revealed a tumor mass which was ultimately the cause of her death.

d. "Epidemiological Follow-Up..." details other workers symptoms as follows: rheumatic-like pains in bones and joints, especially their hips; agonizing pain in the feet; dropped or weak wrist as seen in lead poisoning; extreme sensitivity of the sciatic nerve; dental and jaw trouble expressed as looseness of teeth and decay of gums and jaws; aplastic anemia, necrosis of bones, general sepsis, emaciation and stomach ulcers. In 1928, the first death among the dial painters was reported and radium cancer was listed as the cause of death. A short time after, rare cancers of the paranasal sinuses and air cells in the mastoid bones were discovered among surviving workers.

e. Autopsy of deceased workers revealed unexplained radiolucent areas in mandibles, uneven thickening of bone cortices, changes in trabecular pattern and calcification, histologic changes in soft tissues or individual cells and sub- clinical changes in blood cellular and serum components which were the possible precursors in later diseased conditions.

f. The study concluded that long term surveillance of persons exposed to depleted uranium may be necessary to fulfill the induction process of some tumors and diseases. In addition, it found that even very low body burdens of depleted uranium could result in death.

g. A 1974 study conducted by the Los Alamos Scientific Laboratory of the University of California, Los Alamos, New Mexico, entitled, "Particle Size Distribution of Fragments from Depleted Uranium Penetrators Fired Against Armor Plate Targets," found that firing depleted uranium projectiles promoted fusion. The report concluded "...that these results provide a reasonable estimate of a potential hazard to personnel frequently exposed in test areas or in combat." This introduces another element of DU risk and attendant health effect as depleted uranium fusion and radiation may have been produced in Desert Storm. The consequences of human exposure to atomic/nuclear radiation are well documented and include cancer, leukemia and a general degradation of the autoimmune system.

3. Smoke and chemical pollutants released by the continuous oil well fires:

a. Two studies have been conducted to analyze the chemicals released by the oil combustion of the burning wells. An Environmental Protection Agency task force, headed by Jim Makris, focused on soot levels, carbon monoxide and ozone; chemicals that are dangerous in the short term. The National Toxic Campaign, Boston, Mass., conducted the first laboratory analysis of the smoke and found five toxic hydrocarbon compounds which could result in human health impact.

b. The National Toxic Campaign found 1.4-dichlorobenzene, which can harm the liver, kidneys and respiratory systems; 1.2 dichlorobenzene, which can harm the liver and kidneys; diethyl phthalate and dimethyl phthalate, both of which attack the respiratory system; and naphthalene, which affects the eyes, blood, liver, kidneys and central nervous system.

c. Victor Gordon, a pulmonary specialist at the Veterans' Administration Hospital in Manchester, New Hampshire testified before The Sub-committee on Hospitals and Health Care of the Committee on Veteran's Affair, U. S. House of Representatives, September 21, 1992, in Boston, Mass. He explained that in a less than a year, he had examined 20 Persian Gulf veterans who presented respiratory and/or non-respiratory symptoms. He then detailed a full range of Desert Storm Syndrome symptoms that his patients were suffering from. Linking the smoke from the burning oil wells to their symptoms, he commented, "Consistently, they told about burning skin, oily skin, burning eyes, throat and chest burning." Describing one of his patient's suffering he commented that the man felt as though "somebody poured ground glass" on his chest.

4. Old World Leishmaniasis - a parasitic disease transmitted by the bite of many species of sand fly indigenous to the region. Some 100 species of animals act as reservoir hosts for the sand fly. It is important to note that many Desert Storm soldiers unintentionally increased their likelihood of contracting leishmaniasis by adopting wild dogs and other animals as pets. This disease is common throughout the Middle East and other regions including Africa, Asia, and India; that portion of the Eastern Hemisphere called 'Old World' in ancient history. Leishmaniasis occurring in North and South America is referred to as 'New World' leishmaniasis. Walter Reed Army Hospital has a well established program dedicated to the study of this disease. a. Dr. T. Bektimirov, Assistant Director General of The World Health Organization (WHO) claimed in 1989 that approximately 12 million people worldwide were infected with the disease. He qualified his statement by saying that many cases go unreported, or undiagnosed and this complicates determination of the actual number of cases. WHO acknowledged leishmaniases to be of greater public health importance than was previously recognized. Children and non-indigenous people entering an affected area - such as Desert Storm soldiers from American and other Western nations - are known to suffer more acutely from the disease. Thus far, at least 37 soldiers claim to have contracted leishmaniasis.

b. If left untreated, leishmaniasis can prove fatal.

c. There is no vaccine against leishmaniasis, which is most successfully diagnosed by bone and spleen biopsy.

d. The disease has an incubation period ranging from days to as long as three years and sometimes longer.

e. It can be transmitted by blood transfusion.

f. One tropical disease expert also explained that this disease can be transmitted by a pregnant women to her unborn child.

g. Chemotherapy is the proscribed course of treatment. Pentamidine, meglumine antimoniate, sodium stibogluconate and amphotericin are the drugs in current use. Drug treatment, not always successful, and somewhat costly, ranges from $45.00 to at least $125.00.

h. Leishmaniases takes many forms which can be divided into three types, all of which can strike simultaneously or one at a time:

1. visceral leishmaniasis - which attacks the heart, lungs, liver, spleen, kidneys and intestines. Intercurrent infections, such as pneumonia, dysentery and pulmonary tuberculosis,

may complicate the disease. Visceral leishmaniasis is also called kala azar meaning 'black sickness' because it is known to darken the skin of the face, hands, feet and abdomen in certain of its victims. Twice as many males as females are affected. The incubation period ranges from days to years and the onset of the disease is gradual. Common symptoms are fever, malaise, shivering, drenching sweats, chills, weight loss, anorexia, cough and general signs of malnutrition. Anemia, acute renal damage and severe mucosal hemorrhage may occur in non-indigenous people such as Desert Storm soldiers.

2. post-kala-azar dermal leishmaniases - may occur alone or concurrent with visceral leishmaniases. Most frequently it strikes following visceral form. This form of the disease produces facial redness and chronic facial and body lesions and sores that resemble leprosy. The tongue and lips may be affected and can ulcerate.

3. cutaneous leishmaniasis - clinical features of this strain vary with the species of the parasite and tend to differ between and within regions and also perhaps the genetic predisposition of the patient. This form also produces facial and body lesions which develop slowly, resulting in disfiguring scars. It can be misdiagnosed as leprosy or lupus vulgaris.

i. The insecticides DDT, malathion, fenitrothion, propoxur, deltamethrin, and permethrin have been found effective in controlling the sand fly that carries leishmaniasis.

j. In 1990, according to WHO, Iraq reported 8500 cases of visceral leishmaniasis between 1971 and 1980; then the number levelled off to about 1500 cases per year. The most endemic areas were Central Iraq and Greater Baghdad. Cutaneous leishmaniasis was reported as widespread through the country.

The incidence of both visceral and cutaneous disease were on the rise in Kuwait. Saudi Arabia also reported a widespread incidence of the cutaneous strain throughout the country with 12,000 to 17,000 new cases each year. An increase in the spread of the disease among foreigners was noted. Several hundred cases of visceral leishmaniasis were reported that year in Saudi Arabia.

k. The Pentagon in November 1991 ordered a two-year ban on blood donations from Persian Gulf veterans for fear of spreading the parasitic disease leishmaniasis. The ban squeezed the blood supply at bases worldwide. As a consequence, the blood ban was lifted earlier than anticipated on January 11, 1993.

l. The tiny size of the sandfly makes it difficult to protect against as it can easily penetrate mosquito netting and clothes.

5. Pesticides and insecticides used extensively throughout the war to protect against pestilence.

a. These may include, but are not limited to, DDT, malathion, fenitrorthion, propuxur, deltamethrin, and permethrin. b. Because these chemicals are essentially nerve agents, heavy exposure may result in damage to the central nervous system and varying degrees of neurological disorder.

c. SPECT brain scan examination of 33 patients exposed to a variety of neurotoxins, including pesticides, revealed abnormality in temporal, frontal, occipital and parietal lobes, basal ganglia, thalamus, motorstrip, cerebral hemisphere and the caudate nucleus. (See "Three-Dimensional Brain Metabolic Imaging in Patients with Toxic Encephalopathy" by Thomas J. Callender, Lisa Marrow, Kodanallur Subramanian, Dan Duhon, and Mona Ristovv; presented at the Fourth International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, July 8-11, 1991, Tokyo, Japan.)

d. Symptoms of toxicity include, but are not limited to, fatigue, nausea, impaired concentration, decreased intellectual function, loss of initiative,memory loss, incoherence, loss of night vision, blurry vision, dizziness. Some other symptoms are flu-like symptoms, shortness of breath, upper respiratory infections, increased thirst, myalgia, sense of smell decreased, impotence, hot flashes, weight gain, palpitations, sleep disruption, muscle cramps, bladder spasms, hallucinations, salivation increased, tremors, seizures, voice loss, transiently paralyzed upon wakening and nightmares. (See Callender, et al.)

e. Pesticides are thought to be cancer producing in men and women alike. However, a 1987 study conducted at Hartford Hospital in Connecticut redefined the risk for women. This study found a connection between DDT and other toxic chemicals and breast cancer. DDT is among the class of organochlorines which interfere with the production of estrogen and other hormones that play a role in the development of cancer.

f. In addition, pesticide exposure is widely thought to produce miscarriage, still born birth and birth defects.

6. Allied destruction of Iraqi chemical, nerve and biological warfare weapons resulting in inadvertent exposure to either chemical, nerve or biological warfare agents.

7. Adding to any of these effects, either alone or cumulatively, is the intense Desert Storm electromagnetic environment. The latest research on the thermal and athermal effects of electromagnetic radiation indicate that the broad-spectrum emissions created by the electricity generated to support the mission along with the energy created by the thousands of radio and radar devices deployed in the Persian Gulf theater may act to degrade health. This electropollution can act alone or in concert with other factors to create a synergism that serves to enhance or otherwise alter the effect of drugs or other compounds. There is evidence to suggest that exposure to electromagnetic radiation alone can result in leukemia, non-Hodgkin's lymphoma, cancer, tumor promotion, ocular effects, miscarriage, birth defects, and sterility. As with other pollution, humans are thought to have varying degrees of sensitivity and tolerance to electropollution. In addition to obvious physical effects, ER is thought to affect mood, behavior and task performance. The findings reported are derived from laboratory studies, interviews with researchers and epidemiological studies.

A. Athermal or non-thermal effects are hotly debated in the scientific community studying the consequences of human ER exposure. Contrary to thermal effects, there is neither body fluid or tissue heating with athermal effects. Therefore, there is a lack of consensus as to the mechanism by which athermal effects occur. Many scientists argue that without a clear understanding of the mechanism, there is no strong evidence of consequence. Scientists dedicated to the exploration of the athermal effects of electromagnetic radiation argue otherwise and offer the following information:

a. Researchers report that a few studies on plants and animals report chromosomal aberrations (CAs) or chromosomal breaks.

b. Studies suggest that various combinations of magnetic field strengths and frequencies result in a number of important changes observed at the cell membrane. Cancer promoters are known to interfere with the signals and messages that are being carried across the cell membrane. One effect of this interference is increased cell proliferation and tumor promotion.

c. Electromagnetic radiation (ER) can alter the production of hormones called

neurotransmitters which carry nerve impulses from the brain across the cell membrane. This may have implications in cancer and other diseases.

d. To transmit instructions from the central nervous system, neurotransmitters bind or interact with receptors on the cell membrane. Cancer producing substances are known to increase the number of receptors on a cell. Studies show that electromagnetic radiation exposure increases the numbers of receptors on a cell. e. Receptors following the instructions of the hormones release calcium ions that are stored in the cell membrane. Calcium ions are the primary cell messengers, carrying signals back and forth across the membrane. In this way calcium acts to regulate cell growth and differentiation and tumor production. Studies of ER exposed cells report increases in the calcium flow or 'flux'. These findings suggests changes in cell reproduction consistent with unchecked tumor growth.

f. Calcium flux stimulates enzymes called protein kinases, which also help to regulate cell proliferation. Studies report decreased protein kinase activity in human lymphocyte cells exposed to electromagnetic radiation. (Lymphocytes defend the body against biological agents of disease, chemical invaders and cancerous growths.) In this way ER may suppress the immune system, thus preventing it from performing its function.

g. The enzyme ODC (ornithine decarboxylase) is another enzyme involved in cell growth. Larger amounts of ODC than normal are found to be present during tumor and cancer production. Researchers report increased levels of ODC after ER exposures.

h. Melatonin, an important hormone produced by the pineal gland, is greatly affected by ER exposure. It is melatonin that regulates circadian rhythms, or what is commonly called the 'biological clock'. Circadian rhythms control sleep cycles, moods and task performance. Reduced melatonin levels can contribute to depression, severe mood changes, and certain psychiatric disorders. Decreased levels of melatonin are found in breast, ovarian and prostrate cancer patients. It is theorized that electromagnetic radiation can suppress production of melatonin.

i. ER exposure can cause changes in the blood brain barrier. (The blood-brain barrier is the central nervous system mechanism that prevents toxins from entering brain cells.)

j. Epidemiological studies suggest a connection between power line, communication and radar frequencies and cancer, lymphomas, Hodgkin's disease and leukemia.

k. Desert Storm soldiers can manifest latent symptoms or disease after prolonged exposure to the war's intense and complex electromagnetic environment.

B. Thermal effects are comparatively non-controversial. These are the effects created by electrical heating of body fluids such as those in eyes, testicles, and embryonic sacs. This may cause cataracts and other vision problems, sterility, testicular cancer and miscarriage. Warming sensations, burns, shocks and dry eyes act as preliminary indicators of damage. Damage may manifest in injury either fairly soon after exposure or latently.

May 19 1993 - Patricia Aylwood

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Ms Patricia Axelrod
308 Boyd Ave.
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April 30, 1993

Dear Ms. Axelrod,

Pursuant to our conversation of April 27, regarding the side effects of physostigmine, and similar anticholinesterases including neostigmine (as the methylsulfate) and pyridostigmine, here is the information that you requested.

Physostigmine is a plant-derived alkaloid obtained from the dried ripened seeds of *Physostigma venenosum*. It is the oldest of a class of drugs known as the anticholinesterases. It is also apparently the most potent. Among the anti-cholinesterases are compounds having diverse functional groups including certain quaternary ammonium salts and organophosphates, as well as several naturally-derived or synthetic alkaloids. Those compounds denoted a 'stigmine' are all alkaloids. Neostigmine, for example, is marketed as Prostgmin by Roche Laboratories and is about 60 times less potent than physostigmine.

These drugs block the action of enzymes which hydrolyze acetylcholine. Their administration thus causes increased concentration of this neurotransmitter, a main effector of neuromuscular activity. Inhibition of the cholinesterase enzymes has primary effect of facilitating neuromuscular stimulation. As acetylcholine persists at the neuromuscular junction, muscular strength may be initially increased over short times but will eventually decrease, and paralysis may result.

Agonistic effects in both sympathetic and parasympathetic pathways by the action of anticholinesterases may lead to a mixed variety of inhibitory and stimulatory responses in the central nervous system. Bradycardia, increased gastric secretion, increased sweating and increased salivation are among these effects.

Anticholinesterases are effective topically for many some indications and also may be injected. They may be administered systemically for prevention of post-operative urinary retention and diagnosis and control of myasthenia gravis, for example.

When these drugs are used systemically, the margin in terms of both time and dosage between first indication of toxicity and onset of serious side effects is small indeed. Atropine, when administered early, is effective in blocking many of the adverse side effects of physostigmine toxicity. There is wide variation among patients in response to physostigmine. It's uptake and pharmacolo[illegible]c effect is known to be erratic. Caution is therefore advised. Each patient must be approached as an individual case and adequately monitored during systemic administration. Such advisories are part of the packaging on these drugs.

Signs and symptoms of physostigmine toxicity include confusion, slurred speech and ataxia. Additional adverse effects from excess systemic absorption of these drugs, even after topical administration, may include urinary frequency, urinary urgency, enurisis, hypotension or hypertension, blurred vision, abdominal cramps and involuntary defecation. Excess salivation, nausea and vomiting may also result from acute overdosing.

Anticholinesterases are contraindicated in patients with bronchial asthma, peptic ulcer, hypotension, epilepsy, or Parkinson's disease.

It should be clear from this information that anticholinesterases, especially physostigmine, are potent agents with the potential for serious adverse side effects if improperly administered. Their pharmacological action is known to be erratic and highly variable among individuals. Of the three alkaloid anticholinesterases which you listed, physostigmine appears to be the most potent.

The information that I have provided here is readily available in publications such as the *Physicians Desk Reference* or in books such as *Remington's Pharmaceutical Sciences*. I trust that this will be of some value in you efforts to assist military personnel who may have received this agent.

Sincerely,

Bary W. Wilson, Ph.D.